

(19) World Intellectual Property Organization  
International Bureau



(43) International Publication Date  
9 October 2003 (09.10.2003)

PCT

(10) International Publication Number  
**WO 03/083061 A2**

(51) International Patent Classification<sup>7</sup>:

**C12N**

(21) International Application Number:

**PCT/US03/09072**

(22) International Filing Date:

24 March 2003 (24.03.2003)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

60/367,903 26 March 2002 (26.03.2002) US

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(81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

**Published:**

— without international search report and to be republished upon receipt of that report

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.



**WO 03/083061 A2**

(54) Title: ANTI-TNF ANTIBODIES, COMPOSITIONS, METHODS AND USES

(57) Abstract: The present invention relates to at least one novel anti-TNF antibody, including isolated nucleic acids that encode at least one anti-TNF antibody, TNF, vectors, host cells, transgenic animals or plants, and methods of making and using thereof, including therapeutic compositions, methods and devices.

**ANTI- TNF ANTIBODIES,  
COMPOSITIONS, METHODS AND USES**

**BACKGROUND OF THE INVENTION**

**5 FIELD OF THE INVENTION**

The present invention relates to antibodies, including specified portions or variants, specific for at least one tumor necrosis factor alpha (TNF) protein or fragment thereof, as well as nucleic acids encoding such anti-TNF antibodies, complementary nucleic acids, vectors, host cells, and methods of making and using thereof, including therapeutic formulations, administration and devices.

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**RELATED ART**

TNF alpha is a soluble homotrimer of 17 kD protein subunits (Smith et al., J. Biol. Chem. 262:6951-6954 (1987)). A membrane-bound 26 kD precursor form of TNF also exists (Kriegler et al., Cell 53:45-53 (1988)). For reviews of TNF, see Beutler et al., Nature 320:584 (1986); Old, 15 Science 230:630 (1986); and Le et al., Lab. Invest. 56:234 (1987).

Cells other than monocytes or macrophages also produce TNF alpha. For example, human non-monocytic tumor cell lines produce TNF alpha (Rubin et al., J. Exp. Med. 164:1350 (1986); Spriggs et al., Proc. Natl. Acad. Sci. USA 84:6563 (1987)). CD4+ and CD8+ peripheral blood T lymphocytes and some cultured T and B cell lines (Cuturi et al., J. Exp. Med. 165:1581 (1987); Sung 20 et al., J. Exp. Med. 168:1539 (1988); Turner et al., Eur. J. Immunol. 17:1807-1814 (1987)) also produce TNF alpha.

TNF alpha causes pro-inflammatory actions which result in tissue injury, such as degradation of cartilage and bone (Saklatvala, Nature 322:547-549 (1986); Bertolini, Nature 319:516-518 (1986)), induction of adhesion molecules, inducing procoagulant activity on vascular endothelial cells (Pober 25 et al., J. Immunol. 136:1680 (1986)), increasing the adherence of neutrophils and lymphocytes (Pober et al., J. Immunol. 138:3319 (1987)), and stimulating the release of platelet activating factor from macrophages, neutrophils and vascular endothelial cells (Camussi et al., J. Exp. Med. 166:1390 (1987)).

Recent evidence associates TNF alpha with infections (Cerami et al., Immunol. Today 9:28 30 (1988)), immune disorders, neoplastic pathologies (Oliff et al., Cell 50:555 (1987)), autoimmune pathologies and graft-versus-host pathologies (Piguet et al., J. Exp. Med. 166:1280 (1987)). The association of TNF alpha with cancer and infectious pathologies is often related to the host's

catabolic state. Cancer patients suffer from weight loss, usually associated with anorexia.

The extensive wasting which is associated with cancer, and other diseases, is known as "cachexia" (Kern et al., J. Parent. Enter. Nutr. 12:286-298 (1988)). Cachexia includes progressive weight loss, anorexia, and persistent erosion of lean body mass in response to a malignant growth.

- 5 The cachectic state causes much cancer morbidity and mortality. There is evidence that TNF alpha is involved in cachexia in cancer, infectious pathology, and other catabolic states (see, e.g., Beutler and Cerami, Ann. Rev. Immunol. 7:625-655 (1989)).

TNF alpha is believed to play a central role in gram-negative sepsis and endotoxic shock (Michie et al., Br. J. Surg. 76:670-671 (1989); Debets et al., Second Vienna Shock Forum, p. 10 463-466 (1989); Simpson et al., Crit. Care Clin. 5:27-47 (1989)), including fever, malaise, anorexia, and cachexia. Endotoxin strongly activates monocyte/macrophage production and secretion of TNF alpha and other cytokines (Kornbluth et al., J. Immunol. 137:2585-2591 (1986)). TNF alpha and other monocyte-derived cytokines mediate the metabolic and neurohormonal responses to endotoxin (Michie et al., New Engl. J. Med. 318:1481-1486 (1988)). Endotoxin administration to human 15 volunteers produces acute illness with flu-like symptoms including fever, tachycardia, increased metabolic rate and stress hormone release (Revhaug et al., Arch. Surg. 123:162-170 (1988)). Circulating TNF alpha increases in patients suffering from Gram-negative sepsis (Waage et al., Lancet 1:355-357 (1987); Hammerle et al., Second Vienna Shock Forum, p. 715-718 (1989); Debets et al., Crit. Care Med. 17:489-497 (1989); Calandra et al., J. Infect. Dis. 161:982-987 (1990)).

20 Thus, TNF alpha has been implicated in inflammatory diseases, autoimmune diseases, viral, bacterial and parasitic infections, malignancies, and/or neurogenerative diseases and is a useful target for specific biological therapy in diseases, such as rheumatoid arthritis and Crohn's disease. Beneficial effects in open-label trials with a chimeric monoclonal antibody to TNF alpha (cA2) have been reported with suppression of inflammation and with successful retreatment after relapse in 25 rheumatoid arthritis (Elliott et al., Arthritis Rheum. 36:1681-1690 (1993); and Elliott et al., Lancet 344:1125-1127 (1994)) and in Crohn's disease (Van Dullemen et al., Gastroenterology 109:129-135 (1995)). Beneficial results in a randomized, double-blind, placebo-controlled trial with cA2 have also been reported in rheumatoid arthritis with suppression of inflammation (Elliott et al., Lancet 344:1105-1110 (1994)). Antibodies to a "modulator" material which was characterized as cachectin 30 (later found to be identical to TNF) were disclosed by Cerami et al. (EPO Patent Publication 0212489, March 4, 1987). Such antibodies were said to be useful in diagnostic immunoassays and in therapy of shock in bacterial infections. Rubin et al. (EPO Patent Publication 0218868, April 22, 1987) disclosed monoclonal antibodies to human TNF, the hybridomas secreting such antibodies,

methods of producing such antibodies, and the use of such antibodies in immunoassay of TNF. Yone et al. (EPO Patent Publication 0288088, October 26, 1988) disclosed anti-TNF antibodies, including mAbs, and their utility in immunoassay diagnosis of pathologies, in particular Kawasaki's pathology and bacterial infection. The body fluids of patients with Kawasaki's pathology (infantile acute febrile 5 mucocutaneous lymph node syndrome; Kawasaki, T., Allergy 16:178 (1967); Kawasaki, T., Shonica (Pediatrics) 26:935 (1985)) were said to contain elevated TNF levels which were related to progress of the pathology (Yone et al., *supra*).

Other investigators have described mAbs specific for recombinant human TNF which had neutralizing activity in vitro (Liang, C.-M. et al. (*Biochem. Biophys. Res. Comm.* 137:847-854 10 (1986); Meager, A. et al., *Hybridoma* 6:305-311 (1987); Fendly et al., *Hybridoma* 6:359-369 (1987); Bringman, T.S. et al., *Hybridoma* 6:489-507 (1987); Hirai, M. et al., *J. Immunol. Meth.* 96:57-62 (1987); Moller, A. et al. (*Cytokine* 2:162-169 (1990)). Some of these mAbs were used to map epitopes of human TNF and develop enzyme immunoassays (Fendly et al., *supra*; Hirai et al., *supra*; Moller et al., *supra*) and to assist in the purification of recombinant TNF (Bringman et al., *supra*). 15 However, these studies do not provide a basis for producing TNF neutralizing antibodies that can be used for in vivo diagnostic or therapeutic uses in humans, due to immunogenicity, lack of specificity and/or pharmaceutical suitability.

Neutralizing antisera or mAbs to TNF have been shown in mammals other than man to abrogate adverse physiological changes and prevent death after lethal challenge in experimental 20 endotoxemia and bacteremia. This effect has been demonstrated, e.g., in rodent lethality assays and in primate pathology model systems (Mathison, J.C. et al., *J. Clin. Invest.* 81:1925-1937 (1988); Beutler, B. et al., *Science* 229:869-871 (1985); Tracey, K.J. et al., *Nature* 330:662-664 (1987); Shimamoto, Y. et al., *Immunol. Lett.* 17:311-318 (1988); Silva, A.T. et al., *J. Infect. Dis.* 162:421-427 (1990); Opal, S.M. et al., *J. Infect. Dis.* 161:1148-1152 (1990); Hinshaw, L.B. et al., *Circ. Shock* 25 30:279-292 (1990)).

Putative receptor binding loci of hTNF has been disclosed by Eck and Sprang (*J. Biol. Chem.* 264(29), 17595-17605 (1989), who identified the receptor binding loci of TNF-a as consisting of amino acids 11-13, 37-42, 49-57 and 155-157. PCT application WO91/02078 (priority date of August 7, 1989) discloses TNF ligands which can bind to monoclonal antibodies having the 30 following epitopes: at least one of 1-20, 56-77, and 108-127; at least two of 1-20, 56-77, 108-127 and 138-149; all of 1-18, 58-65, 115-125 and 138-149; all of 1-18, and 108-128; all of 56-79, 110-127 and 135- or 136-155; all of 1-30, 117-128 and 141-153; all of 1-26, 117-128 and 141-153; all of 22-40, 49-96 or -97, 110-127 and 136-153; all of 12-22, 36-45, 96-105 and 132-157; all of both of 1-20 and

76-90; all of 22-40, 69-97, 105-128 and 135-155; all of 22-31 and 146-157; all of 22-40 and 49-98; at least one of 22-40, 49-98 and 69-97, both of 22-40 and 70-87.

Non-human mammalian, chimeric, polyclonal (e.g., anti-sera) and/or monoclonal antibodies (Mabs) and fragments (e.g., proteolytic digestion or fusion protein products thereof) are potential therapeutic agents that are being investigated in some cases to attempt to treat certain diseases. However, such antibodies or fragments can elicit an immune response when administered to humans. Such an immune response can result in an immune complex-mediated clearance of the antibodies or fragments from the circulation, and make repeated administration unsuitable for therapy, thereby reducing the therapeutic benefit to the patient and limiting the readministration of the antibody or fragment. For example, repeated administration of antibodies or fragments comprising non-human portions can lead to serum sickness and/or anaphylaxis. In order to avoid these and other problems, a number of approaches have been taken to reduce the immunogenicity of such antibodies and portions thereof, including chimerization and humanization, as well known in the art. These and other approaches, however, still can result in antibodies or fragments having some immunogenicity, low affinity, low avidity, or with problems in cell culture, scale up, production, and/or low yields. Thus, such antibodies or fragments can be less than ideally suited for manufacture or use as therapeutic proteins.

Accordingly, there is a need to provide anti-TNF antibodies or fragments that overcome one or more of these problems, as well as improvements over known antibodies or fragments thereof.

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#### SUMMARY OF THE INVENTION

The present invention provides isolated human, primate, rodent, mammalian, chimeric, humanized and/or CDR-grafted anti-TNF antibodies, immunoglobulins, cleavage products and other specified portions and variants thereof, as well as anti-TNF antibody compositions, encoding or complementary nucleic acids, vectors, host cells, compositions, formulations, devices, transgenic animals, transgenic plants, and methods of making and using thereof, as described and enabled herein, in combination with what is known in the art.

The present invention also provides at least one isolated anti-TNF antibody as described herein. An antibody according to the present invention includes any protein or peptide containing molecule that comprises at least a portion of an immunoglobulin molecule, such as but not limited to, at least one ligand binding portion (LBP), such as but not limited to, a complementarity determining region (CDR) of a heavy or light chain or a ligand binding portion thereof, a heavy chain or light

chain variable region, a heavy chain or light chain constant region, a framework region, or any portion thereof, that can be incorporated into an antibody of the present invention. An antibody of the invention can include or be derived from any mammal, such as but not limited to a human, a mouse, a rabbit, a rat, a rodent, a primate, or any combination thereof, and the like.

5 The present invention provides, in one aspect, isolated nucleic acid molecules comprising, complementary, or hybridizing to, a polynucleotide encoding specific anti-TNF antibodies, comprising at least one specified sequence, domain, portion or variant thereof. The present invention further provides recombinant vectors comprising said anti-TNF antibody nucleic acid molecules, host cells containing such nucleic acids and/or recombinant vectors, as well as methods of making and/or  
10 using such antibody nucleic acids, vectors and/or host cells.

At least one antibody of the invention binds at least one specified epitope specific to at least one TNF protein, subunit, fragment, portion or any combination thereof. The at least one epitope can comprise at least one antibody binding region that comprises at least one portion of said protein, which epitope is preferably comprised of at least 1-5 amino acids of at least one portion thereof, such  
15 as but not limited to, at least one functional, extracellular, soluble, hydrophilic, external or cytoplasmic domain of said protein, or any portion thereof.

The at least one antibody can optionally comprise at least one specified portion of at least one complementarity determining region (CDR) (e.g., CDR1, CDR2 or CDR3 of the heavy or light chain variable region) and optionally further comprising at least one constant or variable framework region  
20 or any portion thereof. The at least one antibody amino acid sequence can further optionally comprise at least one specified substitution, insertion or deletion as described herein or as known in the art.

The present invention also provides at least one isolated anti-TNF antibody as described herein, wherein the antibody has at least one activity, such as, but not limited to inhibition of TNF-  
25 induced cell adhesion molecules, inhibition of TNF binding to receptor, Arthritic index improvement in mouse model, as known in the art. A(n) anti-TNF antibody can thus be screened for a corresponding activity according to known methods, such as but not limited to, at least one biological activity towards a TNF protein.

The present invention further provides at least one TNF anti-idiotype antibody to at least one  
30 TNF antibody of the present invention. The anti-idiotype antibody includes any protein or peptide containing molecule that comprises at least a portion of an immunoglobulin molecule, such as but not limited to at least one ligand binding portion (LBP), such as but not limited to a complementarity determinng region (CDR) of a heavy or light chain, or a ligand binding portion thereof, a heavy chain

or light chain variable region, a heavy chain or light chain constant region, a framework region, or any portion thereof, that can be incorporated into an antibody of the present invention. An antibody of the invention can include or be derived from any mammal, such as but not limited to a human, a mouse, a rabbit, a rat, a rodent, a primate, and the like.

5       The present invention provides, in one aspect, isolated nucleic acid molecules comprising, complementary, or hybridizing to, a polynucleotide encoding at least one TNF anti-idiotype antibody, comprising at least one specified sequence, domain, portion or variant thereof. The present invention further provides recombinant vectors comprising said TNF anti-idiotype antibody encoding nucleic acid molecules, host cells containing such nucleic acids and/or recombinant vectors, as well as  
10 methods of making and/or using such anti-idiotype antibody nucleic acids, vectors and/or host cells.

The present invention also provides at least one method for expressing at least one anti-TNF antibody, or TNF anti-idiotype antibody, in a host cell, comprising culturing a host cell as described herein under conditions wherein at least one anti-TNF antibody is expressed in detectable and/or recoverable amounts.

15      The present invention also provides at least one composition comprising (a) an isolated anti-TNF antibody encoding nucleic acid and/or antibody as described herein; and (b) a suitable carrier or diluent. The carrier or diluent can optionally be pharmaceutically acceptable, according to known carriers or diluents. The composition can optionally further comprise at least one further compound, protein or composition.

20      The present invention further provides at least one anti-TNF antibody method or composition, for administering a therapeutically effective amount to modulate or treat at least one TNF related condition in a cell, tissue, organ, animal or patient and/or, prior to, subsequent to, or during a related condition, as known in the art and/or as described herein.

25      The present invention also provides at least one composition, device and/or method of delivery of a therapeutically or prophylactically effective amount of at least one anti-TNF antibody, according to the present invention.

30      The present invention further provides at least one anti-TNF antibody method or composition, for diagnosing at least one TNF related condition in a cell, tissue, organ, animal or patient and/or, prior to, subsequent to, or during a related condition, as known in the art and/or as described herein.

The present invention also provides at least one composition, device and/or method of delivery for diagnosing of at least one anti-TNF antibody, according to the present invention.

In one aspect, the present invention provides at least one isolated mammalian anti-TNF

antibody, comprising at least one variable region comprising at least one of SEQ ID NO:7, 8, 15, 16.

In another aspect, the present invention provides at least one isolated mammalian anti-TNF antibody, comprising either (i) all of the heavy chain complementarity determining regions (CDR) amino acid sequences of SEQ ID NOS:1, 2, 3, or 9, 10, 11; or (ii) all of the light chain CDR amino acids sequences of SEQ ID NOS:4, 5, 6 or 12, 13, 14.

In another aspect, the present invention provides at least one isolated mammalian anti-TNF antibody, comprising at least one heavy chain or light chain CDR having the amino acid sequence of at least one of SEQ ID NOS: 1, 2, 3, 4, 5, 6, 9, 10, 11, 12, 13, 14.

10 In other aspect the present invention provides at least one isolated mammalian anti-TNF antibody, comprising at least one human CDR, wherein the antibody specifically binds at least one epitope comprising at least 1-3, to the entire amino acid sequence of SEQ ID NO:17.

The at least one antibody can optionally further at least one of: bind TNF with an affinity of at least one selected from at least  $10^{-9}$  M, at least  $10^{-10}$  M, at least  $10^{-11}$  M, or at least  $10^{-12}$  M; substantially neutralize at least one activity of at least one TNF protein. Also provided is an isolated nucleic acid encoding at least one isolated mammalian anti-TNF antibody; an isolated nucleic acid vector comprising the isolated nucleic acid, and/or a prokaryotic or eukaryotic host cell comprising the isolated nucleic acid. The host cell can optionally be at least one selected from COS-1, COS-7, HEK293, BHK21, CHO, BSC-1, Hep G2, 653, SP2/0, 293, HeLa, myeloma, or lymphoma cells, or any derivative, immortalized or transformed cell thereof. Also provided is a method for producing at least one anti-TNF antibody, comprising translating the antibody encoding nucleic acid under conditions in vitro, in vivo or in situ, such that the TNF antibody is expressed in detectable or recoverable amounts.

Also provided is a composition comprising at least one isolated mammalian anti-TNF antibody and at least one pharmaceutically acceptable carrier or diluent. The composition can optionally further comprise an effective amount of at least one compound or protein selected from at least one of a detectable label or reporter, a TNF antagonist, an antirheumatic, a muscle relaxant, a narcotic, a non-steroid anti-inflammatory drug (NTHE), an analgesic, an anesthetic, a sedative, a local anesthetic, a neuromuscular blocker, an antimicrobial, an antipsoriatic, a corticosteroid, an anabolic steroid, an erythropoietin, an immunization, an immunoglobulin, an immunosuppressive, a growth hormone, a hormone replacement drug, a radiopharmaceutical, an antidepressant, an antipsychotic, a stimulant, an asthma medication, a beta agonist, an inhaled steroid, an epinephrine or

analog, a cytokine, or a cytokine antagonist.

The present invention further provides an anti-idiotype antibody or fragment that specifically binds at least one isolated mammalian anti-TNF antibody of the present invention.

Also provided is a method for diagnosing or treating a TNF related condition in a cell, tissue, 5 organ or animal, comprising

(a) contacting or administering a composition comprising an effective amount of at least one isolated mammalian anti-TNF antibody of the invention with, or to, the cell, tissue, organ or animal. The method can optionally further comprise using an effective amount of 0.001-50 mg/kilogram of the cells, tissue, organ or animal. The method can optionally further comprise using the contacting or 10 administrating by at least one mode selected from parenteral, subcutaneous, intramuscular, intravenous, intrarticular, intrabronchial, intraabdominal, intracapsular, intracartilaginous, intracavitory, intracelial, intracelebellar, intracerebroventricular, intracolic, intracervical, intragastric, intrahepatic, intramyocardial, intraosteal, intrapelvic, intrapericardiac, intraperitoneal, intrapleural, intraprostatic, intrapulmonary, intrarectal, intrarenal, intraretinal, intraspinal, intrasynovial, 15 intrathoracic, intrauterine, intravesical, bolus, vaginal, rectal, buccal, sublingual, intranasal, or transdermal. The method can optionally further comprise administering, prior, concurrently or after the (a) contacting or administering, at least one composition comprising an effective amount of at least one compound or protein selected from at least one of a detectable label or reporter, a TNF antagonist, an antirheumatic, a muscle relaxant, a narcotic, a non-steroid anti-inflammatory drug 20 (NTHE), an analgesic, an anesthetic, a sedative, a local anesthetic, a neuromuscular blocker, an antimicrobial, an antipsoriatic, a corticosteriod, an anabolic steroid, an erythropoietin, an immunization, an immunoglobulin, an immunosuppressive, a growth hormone, a hormone replacement drug, a radiopharmaceutical, an antidepressant, an antipsychotic, a stimulant, an asthma medication, a beta agonist, an inhaled steroid, an epinephrine or analog, a cytokine, or a cytokine 25 antagonist.

Also provided is a medical device, comprising at least one isolated mammalian anti-TNF antibody of the invention, wherein the device is suitable to contacting or administering the at least one anti-TNF antibody by at least one mode selected from parenteral, subcutaneous, intramuscular, intravenous, intrarticular, intrabronchial, intraabdominal, intracapsular, intracartilaginous, 30 intracavitory, intracelial, intracelebellar, intracerebroventricular, intracolic, intracervical, intragastric, intrahepatic, intramyocardial, intraosteal, intrapelvic, intrapericardiac, intraperitoneal, intrapleural, intraprostatic, intrapulmonary, intrarectal, intrarenal, intraretinal, intraspinal, intrasynovial, intrathoracic, intrauterine, intravesical, bolus, vaginal, rectal, buccal, sublingual, intranasal, or

transdermal.

Also provided is an article of manufacture for human pharmaceutical or diagnostic use, comprising packaging material and a container comprising a solution or a lyophilized form of at least one isolated mammalian anti-TNF antibody of the present invention. The article of manufacture can 5 optionally comprise having the container as a component of a parenteral, subcutaneous, intramuscular, intravenous, intrarticular, intrabronchial, intraabdominal, intracapsular, intracartilaginous, intracavitory, intracelial, intracelebellar, intracerebroventricular, intracolic, intracervical, intragastric, intrahepatic, intramyocardial, intraosteal, intrapelvic, intrapericardiac, intraperitoneal, intrapleural, intraprostatic, intrapulmonary, intrarectal, intrarenal, intraretinal, 10 intraspinal, intrasynovial, intrathoracic, intrauterine, intravesical, bolus, vaginal, rectal, buccal, sublingual, intranasal, or transdermal delivery device or system.

Also provided is a method for producing at least one isolated mammalian anti-TNF antibody of the present invention, comprising providing a host cell or transgenic animal or transgenic plant or plant cell capable of expressing in recoverable amounts the antibody. Further provided in the present 15 invention is at least one anti-TNF antibody produced by the above method.

The present invention further provides any invention described herein.

#### DESCRIPTION OF THE FIGURES

**Figures 1A-F:** The MABs indicated in FIG. 1A-F were preincubated with human TNF $\alpha$  for 20 30 minutes and this mixture was added to the WEHI 164 cell media. Plates were then incubated overnight at 37°C, in a 5% CO<sub>2</sub> atmosphere. The final TNF $\alpha$  concentration in the cell media was either 0.2 ng/ml (FIG 1A, C, E) or 0.4 ng/ml (FIG 1B, D, F). The final MAB concentrations in the cell media were as indicated in FIG. 1A-F. Negative controls included cell media alone or cell media with 0.2 ng/ml (FIG. 1A, C, E) or 0.4 ng/ml (FIG. 1B, D, F) TNF $\alpha$ . In FIG. 1E-F the negative control 25 antibody was the CD152 specific ANC152.2/8H5 MAB (ANCELL Corp., Bayport, MN, USA) which was added to the cell media at the final concentrations indicated in FIG. 1E-F. The positive control antibody in FIG. 1E-F was the TNF $\alpha$  specific, human J1D9 MAB (ANCELL Corp., Bayport, MN, USA) which was also added to the final concentrations indicated in FIG. 1E-F.

**Figure 2:** The MABs indicated in FIG. 2 were then preincubated with human TNF $\alpha$  for 30 30 minutes. Next the KYM-1D4 cells were incubated overnight at 37°C in a 5% CO<sub>2</sub> atmosphere with each MAB and human TNF $\alpha$  mixture. The final TNF $\alpha$  concentration in the cell media was 0.2 ng/ml (FIG 2). The final MAB concentrations were as indicated in FIG. 2. The positive control antibody

consisted of the TNF $\alpha$  specific rTNV148B MAB at the concentrations indicated in FIG. 2. Cell viability was determined by adding methylthiazoletetrazolium (MTT) (Sigma-Aldrich Fine Chemicals, St. Louis, MO, USA) to the culture media to a final concentration of 0.5 mg/ml, incubating for 3 hr at 37°C in 5% CO<sub>2</sub> atmosphere, removing the media, adding dimethylsulfoxide (DMSO) (Sigma-Aldrich Fine Chemicals, St. Louis, MO, USA) and measuring A<sub>550-650nm</sub>.

5 **Figure 3A-B:** Determination of the variable region sequences confirmed that the antibodies were of human sequence. Two human light chain sequences, 9C1LC and 9E7LC, were amplified from each clone.

10 **Figure 4:** One HC variable region sequence was amplified from the total RNA of the 9C1.1, 9C1.20, 9E6.6, 9E7.1, 10D1.5, and 11E11.6 hybridomas. This HC sequence was identical in all six hybridoma cell lines and was named 9C1HC (FIG. 4).

15 **Figure 5:** The MABs indicated in FIG. 5 were preincubated with human TNF $\alpha$  for 30 minutes and this mixture was added to the WEHI 164 cell media. Plates were then incubated overnight at 37°C, in a 5% CO<sub>2</sub> atmosphere. The final TNF $\alpha$  concentration in the cell media was 0.2 ng/ml. The final MAB concentrations in the cell media were as indicated in FIG. 5. The positive control antibody in FIG. 5 was the highly potent, human TNF $\alpha$  specific rTNV148B MAB which was added to the final concentrations indicated in FIG. 5. Cell viability was determined by adding MTT to the culture media to a final concentration of 0.5 mg/ml, incubating for 3 hours at 37°C in 5%CO<sub>2</sub> atmosphere, removing the media adding DMSO and measuring absorbance at 550 nm. As seen in 20 FIG. 5 the r9C1A MAB is substantially more potent than the r9C1 MAB and approximately 5 fold more potent than the positive control rTNV148B MAB.

25 **Figure 6:** As seen in FIG. 6 the r9C1A MAB inhibited <sup>125</sup>I labeled TNF $\alpha$  binding to the recombinant P55-sf2 TNF $\alpha$  receptor. In contrast, the r9C1 MAB was unable to prevent the binding of TNF $\alpha$  to the P55-sf2 TNF $\alpha$  receptor as shown in FIG. 6. Additionally, the r9C1A MAB inhibits TNF $\alpha$  binding at an approximately 5 fold lower concentration than the positive control rTNV148B MAB (FIG. 6)

## DESCRIPTION OF THE INVENTION

The present invention provides isolated, recombinant and/or synthetic anti-TNF 30 human, primate, rodent, mammalian, chimeric, humanized or CDR-grafted, antibodies and TNF anti-idiotype antibodies thereto, as well as compositions and encoding nucleic acid molecules comprising

at least one polynucleotide encoding at least one anti-TNF antibody or anti-idiotype antibody. The present invention further includes, but is not limited to, methods of making and using such nucleic acids and antibodies and anti-idiotype antibodies, including diagnostic and therapeutic compositions, methods and devices.

5 As used herein, an "anti-tumor necrosis factor alpha antibody," "anti-TNF antibody," "anti-TNF antibody portion," or "anti-TNF antibody fragment" and/or "anti-TNF antibody variant" and the like include any protein or peptide containing molecule that comprises at least a portion of an immunoglobulin molecule, such as but not limited to at least one complementarity determining region (CDR) of a heavy or light chain or a ligand binding portion thereof, a heavy chain or light chain  
10 variable region, a heavy chain or light chain constant region, a framework region, or any portion thereof, or at least one portion of an TNF receptor or binding protein, which can be incorporated into an antibody of the present invention. Such antibody optionally further affects a specific ligand, such as but not limited to where such antibody modulates, decreases, increases, antagonizes, agonizes, mitigates, alleviates, blocks, inhibits, abrogates and/or interferes with at least one TNF activity or  
15 binding, or with TNF receptor activity or binding, *in vitro*, *in situ* and/or *in vivo*. As a non-limiting example, a suitable anti-TNF antibody, specified portion or variant of the present invention can bind at least one TNF, or specified portions, variants or domains thereof. A suitable anti-TNF antibody, specified portion, or variant can also optionally affect at least one of TNF activity or function, such as but not limited to, RNA, DNA or protein synthesis, TNF release, TNF receptor signaling, membrane  
20 TNF cleavage, TNF activity, TNF production and/or synthesis. The term "antibody" is further intended to encompass antibodies, digestion fragments, specified portions and variants thereof, including antibody mimetics or comprising portions of antibodies that mimic the structure and/or function of an antibody or specified fragment or portion thereof, including single chain antibodies and fragments thereof. Functional fragments include antigen-binding fragments that bind to a  
25 mammalian TNF. For example, antibody fragments capable of binding to TNF or portions thereof, including, but not limited to Fab (e.g., by papain digestion), Fab' (e.g., by pepsin digestion and partial reduction) and F(ab')<sub>2</sub> (e.g., by pepsin digestion), facb (e.g., by plasmin digestion), pFc' (e.g., by pepsin or plasmin digestion), Fd (e.g., by pepsin digestion, partial reduction and reaggregation), Fv or scFv (e.g., by molecular biology techniques) fragments, are encompassed by the invention (see, e.g.,  
30 Colligan, Immunology, supra).

Such fragments can be produced by enzymatic cleavage, synthetic or recombinant techniques, as known in the art and/or as described herein. Antibodies can also be produced in a variety of truncated forms using antibody genes in which one or more stop codons have been introduced

upstream of the natural stop site. For example, a combination gene encoding a F(ab')<sub>2</sub> heavy chain portion can be designed to include DNA sequences encoding the CH<sub>1</sub> domain and/or hinge region of the heavy chain. The various portions of antibodies can be joined together chemically by conventional techniques, or can be prepared as a contiguous protein using genetic engineering  
5 techniques.

As used herein, the term "human antibody" refers to an antibody in which substantially every part of the protein (e.g., CDR, framework, C<sub>L</sub>, C<sub>H</sub> domains (e.g., C<sub>H</sub>1, C<sub>H</sub>2, C<sub>H</sub>3), hinge, (V<sub>L</sub>, V<sub>H</sub>)) is substantially non-immunogenic in humans, with only minor sequence changes or variations.  
Similarly, antibodies designated primate (monkey, baboon, chimpanzee, etc.), rodent (mouse, rat,  
10 rabbit, guinea pig, hamster, and the like) and other mammals designate such species, sub-genus, genus, sub-family, family specific antibodies. Further, chimeric antibodies of the invention can include any combination of the above. Such changes or variations optionally and preferably retain or reduce the immunogenicity in humans or other species relative to non-modified antibodies. Thus, a  
15 human antibody is distinct from a chimeric or humanized antibody. It is pointed out that a human antibody can be produced by a non-human animal or prokaryotic or eukaryotic cell that is capable of expressing functionally rearranged human immunoglobulin (e.g., heavy chain and/or light chain) genes. Further, when a human antibody is a single chain antibody, it can comprise a linker peptide that is not found in native human antibodies. For example, an Fv can comprise a linker peptide, such as two to about eight glycine or other amino acid residues, which connects the variable region of the  
20 heavy chain and the variable region of the light chain. Such linker peptides are considered to be of human origin.

Bispecific, heterospecific, heteroconjugate or similar antibodies can also be used that are monoclonal, preferably human or humanized, antibodies that have binding specificities for at least two different antigens. In the present case, one of the binding specificities is for at least one TNF  
25 protein, the other one is for any other antigen. Methods for making bispecific antibodies are known in the art. Traditionally, the recombinant production of bispecific antibodies is based on the co-expression of two immunoglobulin heavy chain-light chain pairs, where the two heavy chains have different specificities (Milstein and Cuello, Nature 305:537 (1983)). Because of the random assortment of immunoglobulin heavy and light chains, these hybridomas (quadromas) produce a  
30 potential mixture of 10 different antibody molecules, of which only one has the correct bispecific structure. The purification of the correct molecule, which is usually done by affinity chromatography steps, is rather cumbersome, and the product yields are low. Similar procedures are disclosed, e.g., in WO 93/08829, US Patent Nos, 6210668, 6193967, 6132992, 6106833, 6060285, 6037453, 6010902,

5989530, 5959084, 5959083, 5932448, 5833985, 5821333, 5807706, 5643759, 5601819, 5582996, 5496549, 4676980, WO 91/00360, WO 92/00373, EP 03089, Traunecker et al., EMBO J. 10:3655 (1991), Suresh et al., Methods in Enzymology 121:210 (1986), each entirely incorporated herein by reference.

5       Anti-TNF antibodies (also termed TNF antibodies) useful in the methods and compositions of the present invention can optionally be characterized by high affinity binding to TNF and optionally and preferably having low toxicity. In particular, an antibody, specified fragment or variant of the invention, where the individual components, such as the variable region, constant region and framework, individually and/or collectively, optionally and preferably possess low 10 immunogenicity, is useful in the present invention. The antibodies that can be used in the invention are optionally characterized by their ability to treat patients for extended periods with measurable alleviation of symptoms and low and/or acceptable toxicity. Low or acceptable immunogenicity and/or high affinity, as well as other suitable properties, can contribute to the therapeutic results achieved. "Low immunogenicity" is defined herein as raising significant HAHA, HACA or HAMA 15 responses in less than about 75%, or preferably less than about 50% of the patients treated and/or raising low titres in the patient treated (less than about 300, preferably less than about 100 measured with a double antigen enzyme immunoassay) (Elliott et al., *Lancet* 344:1125-1127 (1994), entirely incorporated herein by reference).

*Utility*

20     The isolated nucleic acids of the present invention can be used for production of at least one anti-TNF antibody or specified variant thereof, which can be used to measure or effect in a cell, tissue, organ or animal (including mammals and humans), to diagnose, monitor, modulate, treat, alleviate, help prevent the incidence of, or reduce the symptoms of, at least one TNF condition, selected from, but not limited to, at least one of an immune disorder or disease, a cardiovascular 25 disorder or disease, an infectious, malignant, and/or neurologic disorder or disease, or other known or specified TNF related condition.

Such a method can comprise administering an effective amount of a composition or a pharmaceutical composition comprising at least one anti-TNF antibody to a cell, tissue, organ, animal or patient in need of such modulation, treatment, alleviation, prevention, or reduction in symptoms, 30 effects or mechanisms. The effective amount can comprise an amount of about 0.001 to 500 mg/kg per single (e.g., bolus), multiple or continuous administration, or to achieve a serum concentration of 0.01-5000 µg/ml serum concentration per single, multiple, or continuous administration, or any

effective range or value therein, as done and determined using known methods, as described herein or known in the relevant arts.

*Citations*

- All publications or patents cited herein are entirely incorporated herein by reference as they show the state of the art at the time of the present invention and/or to provide description and enablement of the present invention. Publications refer to any scientific or patent publications, or any other information available in any media format, including all recorded, electronic or printed formats.
- The following references are entirely incorporated herein by reference: Ausubel, et al., ed., Current Protocols in Molecular Biology, John Wiley & Sons, Inc., NY, NY (1987-2001); Sambrook, et al., Molecular Cloning: A Laboratory Manual, 2<sup>nd</sup> Edition, Cold Spring Harbor, NY (1989); Harlow and Lane, antibodies, a Laboratory Manual, Cold Spring Harbor, NY (1989); Colligan, et al., eds., Current Protocols in Immunology, John Wiley & Sons, Inc., NY (1994-2001); Colligan et al., Current Protocols in Protein Science, John Wiley & Sons, NY, NY, (1997-2001).

*Antibodies of the Present Invention*

- At least one anti-TNF antibody of the present invention can be optionally produced by a cell line, a mixed cell line, an immortalized cell or clonal population of immortalized cells, as well known in the art. See, e.g., Ausubel, et al., ed., Current Protocols in Molecular Biology, John Wiley & Sons, Inc., NY, NY (1987-2001); Sambrook, et al., Molecular Cloning: A Laboratory Manual, 2<sup>nd</sup> Edition, Cold Spring Harbor, NY (1989); Harlow and Lane, antibodies, a Laboratory Manual, Cold Spring Harbor, NY (1989); Colligan, et al., eds., Current Protocols in Immunology, John Wiley & Sons, Inc., NY (1994-2001); Colligan et al., Current Protocols in Protein Science, John Wiley & Sons, NY, NY, (1997-2001), each entirely incorporated herein by reference.

Human antibodies that are specific for human TNF proteins or fragments thereof can be raised against an appropriate immunogenic antigen, such as isolated and/or TNF protein or a portion thereof (including synthetic molecules, such as synthetic peptides). Other specific or general mammalian antibodies can be similarly raised. Preparation of immunogenic antigens, and monoclonal antibody production can be performed using any suitable technique.

- In one approach, a hybridoma is produced by fusing a suitable immortal cell line (e.g., a myeloma cell line such as, but not limited to, Sp2/0, Sp2/0-AG14, NSO, NS1, NS2, AE-1, L.5, >243, P3X63Ag8.653, Sp2 SA3, Sp2 MAI, Sp2 SS1, Sp2 SA5, U937, MLA 144, ACT IV, MOLT4, DA-1, JURKAT, WEHI, K-562, COS, RAJI, NIH 3T3, HL-60, MLA 144, NAMAIWA, NEURO 2A, or the like, or heteromylomas, fusion products thereof, or any cell or fusion cell derived therefrom, or any other suitable cell line as known in the art. See, e.g., www.atcc.org, www.lifetech.com., and the like,

- with antibody producing cells, such as, but not limited to, isolated or cloned spleen, peripheral blood, lymph, tonsil, or other immune or B cell containing cells, or any other cells expressing heavy or light chain constant or variable or framework or CDR sequences, either as endogenous or heterologous nucleic acid, as recombinant or endogenous, viral, bacterial, algal, prokaryotic, amphibian, insect, 5 reptilian, fish, mammalian, rodent, equine, ovine, goat, sheep, primate, eukaryotic, genomic DNA, cDNA, rDNA, mitochondrial DNA or RNA, chloroplast DNA or RNA, hnRNA, mRNA, tRNA, single, double or triple stranded, hybridized, and the like or any combination thereof. See, e.g., Ausubel, *supra*, and Colligan, *Immunology, supra*, chapter 2, entirely incorporated herein by reference.
- 10 Antibody producing cells can also be obtained from the peripheral blood or, preferably the spleen or lymph nodes, of humans or other suitable animals that have been immunized with the antigen of interest. Any other suitable host cell can also be used for expressing heterologous or endogenous nucleic acid encoding an antibody, specified fragment or variant thereof, of the present invention. The fused cells (hybridomas) or recombinant cells can be isolated using selective culture 15 conditions or other suitable known methods, and cloned by limiting dilution or cell sorting, or other known methods. Cells which produce antibodies with the desired specificity can be selected by a suitable assay (e.g., ELISA).
- Other suitable methods of producing or isolating antibodies of the requisite specificity can be used, including, but not limited to, methods that select recombinant antibody from a peptide or 20 protein library (e.g., but not limited to, a bacteriophage, ribosome, oligonucleotide, RNA, cDNA, or the like, display library; e.g., as available from Cambridge antibody Technologies, Cambridgeshire, UK; MorphoSys, Martinsreid/Planegg, DE; Biovation, Aberdeen, Scotland, UK; BioInvent, Lund, Sweden; Dyax Corp., Enzon, Affymax/Biosite; Xoma, Berkeley, CA; Ixsys. See, e.g., EP 368,684, PCT/GB91/01134; PCT/GB92/01755; PCT/GB92/002240; PCT/GB92/00883; PCT/GB93/00605; US 25 08/350260(5/12/94); PCT/GB94/01422; PCT/GB94/02662; PCT/GB97/01835; (CAT/MRC); WO90/14443; WO90/14424; WO90/14430; PCT/US94/1234; WO92/18619; WO96/07754; (Scripps); WO96/13583, WO97/08320 (MorphoSys); WO95/16027 (BioInvent); WO88/06630; WO90/3809 (Dyax); US 4,704,692 (Enzon); PCT/US91/02989 (Affymax); WO89/06283; EP 371 998; EP 550 400; (Xoma); EP 229 046; PCT/US91/07149 (Ixsys); or stochastically generated 30 peptides or proteins - US 5723323, 5763192, 5814476, 5817483, 5824514, 5976862, WO 86/05803, EP 590 689 (Ixsys, now Applied Molecular Evolution (AME), each entirely incorporated herein by reference) or that rely upon immunization of transgenic animals (e.g., SCID mice, Nguyen et al., *Microbiol. Immunol.* 41:901-907 (1997); Sandhu et al., *Crit. Rev. Biotechnol.* 16:95-118 (1996);

Eren et al., Immunol. 93:154-161 (1998), each entirely incorporated by reference as well as related patents and applications) that are capable of producing a repertoire of human antibodies, as known in the art and/or as described herein. Such techniques, include, but are not limited to, ribosome display (Hanes et al., Proc. Natl. Acad. Sci. USA, 94:4937-4942 (May 1997); Hanes et al., Proc. Natl. Acad. Sci. USA, 95:14130-14135 (Nov. 1998)); single cell antibody producing technologies (e.g., selected lymphocyte antibody method ("SLAM") (US pat. No. 5,627,052, Wen et al., J. Immunol. 17:887-892 (1987); Babcock et al., Proc. Natl. Acad. Sci. USA 93:7843-7848 (1996)); gel microdroplet and flow cytometry (Powell et al., Biotechnol. 8:333-337 (1990); One Cell Systems, Cambridge, MA; Gray et al., J. Imm. Meth. 182:155-163 (1995); Kenny et al., Bio/Technol. 13:787-790 (1995)); B-cell selection (Steenbakkers et al., Molec. Biol. Reports 19:125-134 (1994); Jonak et al., Progress Biotech, Vol. 5, In Vitro Immunization in Hybridoma Technology, Borrebaeck, ed., Elsevier Science Publishers B.V., Amsterdam, Netherlands (1988)).

Methods for engineering or humanizing non-human or human antibodies can also be used and are well known in the art. Generally, a humanized or engineered antibody has one or more amino acid residues from a source which is non-human, e.g., but not limited to mouse, rat, rabbit, non-human primate or other mammal. These human amino acid residues are often referred to as "import" residues, which are typically taken from an "import" variable, constant or other domain of a known human sequence. Known human Ig sequences are disclosed, e.g.,  
[www.ncbi.nlm.nih.gov/entrez/query.fcgi](http://www.ncbi.nlm.nih.gov/entrez/query.fcgi); [www.atcc.org/phage/hdb.html](http://www.atcc.org/phage/hdb.html); [www.sciquest.com/](http://www.sciquest.com/);  
20 [www.abcam.com/](http://www.abcam.com/); [www.antibodyresource.com/onlinecomp.html](http://www.antibodyresource.com/onlinecomp.html);  
[www.public.iastate.edu/~pedro/research\\_tools.html](http://www.public.iastate.edu/~pedro/research_tools.html); [www.mgen.uni-heidelberg.de/SD/IT/IT.html](http://www.mgen.uni-heidelberg.de/SD/IT/IT.html);  
[www.whfreeman.com/immunology/CH05/kuby05.htm](http://www.whfreeman.com/immunology/CH05/kuby05.htm);  
[www.library.thinkquest.org/12429/Immune/Antibody.html](http://www.library.thinkquest.org/12429/Immune/Antibody.html);  
[www.hhmi.org/grants/lectures/1996/vlab/](http://www.hhmi.org/grants/lectures/1996/vlab/); [www.path.cam.ac.uk/~mrc7/mikeimages.html](http://www.path.cam.ac.uk/~mrc7/mikeimages.html);  
25 [www.antibodyresource.com/](http://www.antibodyresource.com/);  
[mcb.harvard.edu/BioLinks/Immunology.html](http://mcb.harvard.edu/BioLinks/Immunology.html).[www.immunologylink.com/](http://www.immunologylink.com/);  
[pathbox.wustl.edu/~hcenter/index.html](http://pathbox.wustl.edu/~hcenter/index.html); [www.biotech.ufl.edu/~hcl/](http://www.biotech.ufl.edu/~hcl/);  
[www.pebio.com/pa/340913/340913.html](http://www.pebio.com/pa/340913/340913.html); [www.nal.usda.gov/awic/pubs/antibody/](http://www.nal.usda.gov/awic/pubs/antibody/);  
[www.m.ehime-u.ac.jp/~yasuhito/Elisa.html](http://www.m.ehime-u.ac.jp/~yasuhito/Elisa.html); [www.biodesign.com/table.asp](http://www.biodesign.com/table.asp);  
30 [www.icnet.uk/axp/facs/davies/links.html](http://www.icnet.uk/axp/facs/davies/links.html); [www.biotech.ufl.edu/~fccl/protocol.html](http://www.biotech.ufl.edu/~fccl/protocol.html); [www.isac-net.org/sites\\_geo.html](http://www.isac-net.org/sites_geo.html); [aximt1.imt.uni-marburg.de/~rek/AEPStart.html](http://aximt1.imt.uni-marburg.de/~rek/AEPStart.html);  
[baserv.uci.kun.nl/~jraats/links1.html](http://baserv.uci.kun.nl/~jraats/links1.html); [www.recab.uni-hd.de/immuno.bme.nwu.edu/](http://www.recab.uni-hd.de/immuno.bme.nwu.edu/); [www.mrc-](http://www.mrc-)

cpe.cam.ac.uk/imt-doc/public/INTRO.html; www.ibt.unam.mx/vir/V\_mice.html; imgt.cnusc.fr:8104/; www.biochem.ucl.ac.uk/~martin/abs/index.html; antibody.bath.ac.uk/; abgen.cvm.tamu.edu/lab/wwwabgen.html; www.unizh.ch/~honegger/AHOseminar/Slide01.html; www.cryst.bbk.ac.uk/~ubcg07s/; www.nimr.mrc.ac.uk/CC/ccaewg/ccaewg.htm;

5      www.path.cam.ac.uk/~mrc7/humanisation/TAHHP.html;

www.ibt.unam.mx/vir/structure/stat\_aim.html; www.biosci.missouri.edu/smithgp/index.html;

www.cryst.bioc.cam.ac.uk/~fmolina/Web-pages/Pept/spottech.html; www.jerini.de/fr\_products.htm;

www.patents.ibm.com/ibm.html.Kabat et al., Sequences of Proteins of Immunological Interest, U.S. Dept. Health (1983), each entirely incorporated herein by reference.

10     Such imported sequences can be used to reduce immunogenicity or reduce, enhance or modify binding, affinity, on-rate, off-rate, avidity, specificity, half-life, or any other suitable characteristic, as known in the art. Generally part or all of the non-human or human CDR sequences are maintained while the non-human sequences of the variable and constant regions are replaced with human or other amino acids. antibodies can also optionally be humanized with retention of high

15     affinity for the antigen and other favorable biological properties. To achieve this goal, humanized antibodies can be optionally prepared by a process of analysis of the parental sequences and various conceptual humanized products using three-dimensional models of the parental and humanized sequences. Three-dimensional immunoglobulin models are commonly available and are familiar to those skilled in the art. Computer programs are available which illustrate and display probable three-

20     dimensional conformational structures of selected candidate immunoglobulin sequences. Inspection of these displays permits analysis of the likely role of the residues in the functioning of the candidate immunoglobulin sequence, i.e., the analysis of residues that influence the ability of the candidate immunoglobulin to bind its antigen. In this way, FR residues can be selected and combined from the consensus and import sequences so that the desired antibody characteristic, such as increased affinity

25     for the target antigen(s), is achieved. In general, the CDR residues are directly and most substantially involved in influencing antigen binding. Humanization or engineering of antibodies of the present invention can be performed using any known method, such as but not limited to those described in, Winter (Jones et al., Nature 321:522 (1986); Riechmann et al., Nature 332:323 (1988); Verhoeyen et al., Science 239:1534 (1988)), Sims et al., J. Immunol. 151: 2296 (1993); Chothia and Lesk, J. Mol. Biol. 196:901 (1987), Carter et al., Proc. Natl. Acad. Sci. U.S.A. 89:4285 (1992); Presta et al., J.

30     Immunol. 151:2623 (1993), US patent Nos: 5723323, 5976862, 5824514, 5817483, 5814476, 5763192, 5723323, 5,766886, 5714352, 6204023, 6180370, 5693762, 5530101, 5585089, 5225539; 4816567, PCT/: US98/16280, US96/18978, US91/09630, US91/05939, US94/01234, GB89/01334,

GB91/01134, GB92/01755; WO90/14443, WO90/14424, WO90/14430, EP 229246, each entirely incorporated herein by reference, included references cited therein.

The anti-TNF antibody can also be optionally generated by immunization of a transgenic animal (e.g., mouse, rat, hamster, non-human primate, and the like) capable of producing a repertoire of human antibodies, as described herein and/or as known in the art. Cells that produce a human anti-TNF antibody can be isolated from such animals and immortalized using suitable methods, such as the methods described herein.

Transgenic mice that can produce a repertoire of human antibodies that bind to human antigens can be produced by known methods (e.g., but not limited to, U.S. Pat. Nos: 5,770,428, 10 5,569,825, 5,545,806, 5,625,126, 5,625,825, 5,633,425, 5,661,016 and 5,789,650 issued to Lonberg *et al.*; Jakobovits *et al.* WO 98/50433, Jakobovits *et al.* WO 98/24893, Lonberg *et al.* WO 98/24884, Lonberg *et al.* WO 97/13852, Lonberg *et al.* WO 94/25585, Kucherlapate *et al.* WO 96/34096, Kucherlapate *et al.* EP 0463 151 B1, Kucherlapate *et al.* EP 0710 719 A1, Surani *et al.* US. Pat. No. 5,545,807, Bruggemann *et al.* WO 90/04036, Bruggemann *et al.* EP 0438 474 B1, Lonberg *et al.* EP 15 0814 259 A2, Lonberg *et al.* GB 2 272 440 A, Lonberg *et al.* *Nature* 368:856-859 (1994), Taylor *et al.*, *Int. Immunol.* 6(4):579-591 (1994), Green *et al.*, *Nature Genetics* 7:13-21 (1994), Mendez *et al.*, *Nature Genetics* 15:146-156 (1997), Taylor *et al.*, *Nucleic Acids Research* 20(23):6287-6295 (1992), Tuailon *et al.*, *Proc Natl Acad Sci USA* 90(8):3720-3724 (1993), Lonberg *et al.*, *Int Rev Immunol* 13(1):65-93 (1995) and Fishwald *et al.*, *Nat Biotechnol* 14(7):845-851 (1996), which are each entirely 20 incorporated herein by reference). Generally, these mice comprise at least one transgene comprising DNA from at least one human immunoglobulin locus that is functionally rearranged, or which can undergo functional rearrangement. The endogenous immunoglobulin loci in such mice can be disrupted or deleted to eliminate the capacity of the animal to produce antibodies encoded by endogenous genes.

Screening antibodies for specific binding to similar proteins or fragments can be conveniently achieved using peptide display libraries. This method involves the screening of large collections of peptides for individual members having the desired function or structure. Antibody screening of peptide display libraries is well known in the art. The displayed peptide sequences can be from 3 to 5000 or more amino acids in length, frequently from 5-100 amino acids long, and often from about 8 to 25 25 amino acids long. In addition to direct chemical synthetic methods for generating peptide libraries, several recombinant DNA methods have been described. One type involves the display of a peptide sequence on the surface of a bacteriophage or cell. Each bacteriophage or cell contains the nucleotide sequence encoding the particular displayed peptide sequence. Such methods are described in PCT

Patent Publication Nos. 91/17271, 91/18980, 91/19818, and 93/08278. Other systems for generating libraries of peptides have aspects of both in vitro chemical synthesis and recombinant methods. See, PCT Patent Publication Nos. 92/05258, 92/14843, and 96/19256. See also, U.S. Patent Nos. 5,658,754; and 5,643,768. Peptide display libraries, vector, and screening kits are commercially available from 5 such suppliers as Invitrogen (Carlsbad, CA), and Cambridge antibody Technologies (Cambridgeshire, UK). See, e.g., U.S. Pat. Nos. 4704692, 4939666, 4946778, 5260203, 5455030, 5518889, 5534621, 10 5656730, 5763733, 5767260, 5856456, assigned to Enzon; 5223409, 5403484, 5571698, 5837500, assigned to Dyax, 5427908, 5580717, assigned to Affymax; 5885793, assigned to Cambridge antibody Technologies; 5750373, assigned to Genentech, 5618920, 5595898, 5576195, 5698435, 5693493, 15 5698417, assigned to Xoma, Colligan, *supra*; Ausubel, *supra*; or Sambrook, *supra*, each of the above patents and publications entirely incorporated herein by reference.

Antibodies of the present invention can also be prepared using at least one anti-TNF antibody encoding nucleic acid to provide transgenic animals or mammals, such as goats, cows, horses, sheep, and the like, that produce such antibodies in their milk. Such animals can be provided using known 15 methods. See, e.g., but not limited to, US patent nos. 5,827,690; 5,849,992; 4,873,316; 5,849,992; 5,994,616; 5,565,362; 5,304,489, and the like, each of which is entirely incorporated herein by reference.

Antibodies of the present invention can additionally be prepared using at least one anti-TNF antibody encoding nucleic acid to provide transgenic plants and cultured plant cells (e.g., but not 20 limited to tobacco and maize) that produce such antibodies, specified portions or variants in the plant parts or in cells cultured therefrom. As a non-limiting example, transgenic tobacco leaves expressing recombinant proteins have been successfully used to provide large amounts of recombinant proteins, e.g., using an inducible promoter. See, e.g., Cramer et al., *Curr. Top. Microbiol. Immunol.* 240:95-118 (1999) and references cited therein. Also, transgenic maize have been used to express 25 mammalian proteins at commercial production levels, with biological activities equivalent to those produced in other recombinant systems or purified from natural sources. See, e.g., Hood et al., *Adv. Exp. Med. Biol.* 464:127-147 (1999) and references cited therein. antibodies have also been produced in large amounts from transgenic plant seeds including antibody fragments, such as single chain antibodies (scFv's), including tobacco seeds and potato tubers. See, e.g., Conrad et al., *Plant 30 Mol. Biol.* 38:101-109 (1998) and reference cited therein. Thus, antibodies of the present invention can also be produced using transgenic plants, according to know methods. See also, e.g., Fischer et al., *Biotechnol. Appl. Biochem.* 30:99-108 (Oct., 1999), Ma et al., *Trends Biotechnol.* 13:522-7 (1995); Ma et al., *Plant Physiol.* 109:341-6 (1995); Whitelam et al., *Biochem. Soc. Trans.* 22:940-944

(1994); and references cited therein. See, also generally for plant expression of antibodies, but not limited to, Each of the above references is entirely incorporated herein by reference.

The antibodies of the invention can bind human TNF with a wide range of affinities ( $K_D$ ). In a preferred embodiment, at least one human mAb of the present invention can optionally bind human TNF with high affinity. For example, a human mAb can bind human TNF with a  $K_D$  equal to or less than about  $10^{-7}$  M, such as but not limited to, 0.1-9.9 (or any range or value therein)  $\times 10^{-7}$ ,  $10^{-8}$ ,  $10^{-9}$ ,  $10^{-10}$ ,  $10^{-11}$ ,  $10^{-12}$ ,  $10^{-13}$  or any range or value therein.

The affinity or avidity of an antibody for an antigen can be determined experimentally using any suitable method. (See, for example, Berzofsky, *et al.*, "Antibody-Antigen Interactions," In 10 *Fundamental Immunology*, Paul, W. E., Ed., Raven Press: New York, NY (1984); Kuby, Janis *Immunology*, W. H. Freeman and Company: New York, NY (1992); and methods described herein). The measured affinity of a particular antibody-antigen interaction can vary if measured under different conditions (e.g., salt concentration, pH). Thus, measurements of affinity and other antigen-binding parameters (e.g.,  $K_D$ ,  $K_a$ ,  $K_d$ ) are preferably made with standardized solutions of antibody and 15 antigen, and a standardized buffer, such as the buffer described herein.

#### Nucleic Acid Molecules

Using the information provided herein, such as the nucleotide sequences encoding at least 70-100% of the contiguous amino acids of at least one of SEQ ID NOS:1, 2, 3, 4, 5, 6, 7; 8, 9, 10, 11, 12, 13, 14, 15, 16, specified fragments, variants or consensus sequences thereof, or a deposited vector 20 comprising at least one of these sequences, a nucleic acid molecule of the present invention encoding at least one anti-TNF antibody can be obtained using methods described herein or as known in the art.

Nucleic acid molecules of the present invention can be in the form of RNA, such as mRNA, hnRNA, tRNA or any other form, or in the form of DNA, including, but not limited to, cDNA and genomic DNA obtained by cloning or produced synthetically, or any combinations thereof. The 25 DNA can be triple-stranded, double-stranded or single-stranded, or any combination thereof. Any portion of at least one strand of the DNA or RNA can be the coding strand, also known as the sense strand, or it can be the non-coding strand, also referred to as the anti-sense strand.

Isolated nucleic acid molecules of the present invention can include nucleic acid molecules comprising an open reading frame (ORF), optionally with one or more introns, e.g., but not limited 30 to, at least one specified portion of at least one CDR, as CDR1, CDR2 and/or CDR3 of at least one heavy chain (e.g., SEQ ID NOS:1-3, 9-11) or light chain (e.g., SEQ ID NOS: 4-6, 12-14); nucleic acid molecules comprising the coding sequence for an anti-TNF antibody or variable region (e.g., SEQ ID NOS:7,8, 15, 16); and nucleic acid molecules which comprise a nucleotide sequence substantially

different from those described above but which, due to the degeneracy of the genetic code, still encode at least one anti-TNF antibody as described herein and/or as known in the art. Of course, the genetic code is well known in the art. Thus, it would be routine for one skilled in the art to generate such degenerate nucleic acid variants that code for specific anti-TNF antibodies of the present invention. See, e.g., Ausubel, et al., *supra*, and such nucleic acid variants are included in the present invention. Non-limiting examples of isolated nucleic acid molecules of the present invention include those corresponding to non-limiting examples of a nucleic acid encoding, respectively, HC CDR1, HC CDR2, HC CDR3, LC CDR1, LC CDR2, LC CDR3, HC variable region and LC variable region, such as but not limited to those encoding SEQ ID NOS:1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16.

In another aspect, the invention provides isolated nucleic acid molecules encoding a(n) anti-TNF antibody having an amino acid sequence as encoded by the nucleic acid contained in the plasmid deposited as designated clone names \_\_\_\_\_ and ATCC Deposit Nos. \_\_\_\_\_, respectively, deposited on \_\_\_\_\_.

As indicated herein, nucleic acid molecules of the present invention which comprise a nucleic acid encoding an anti-TNF antibody can include, but are not limited to, those encoding the amino acid sequence of an antibody fragment, by itself; the coding sequence for the entire antibody or a portion thereof; the coding sequence for an antibody, fragment or portion, as well as additional sequences, such as the coding sequence of at least one signal leader or fusion peptide, with or without the aforementioned additional coding sequences, such as at least one intron, together with additional, non-coding sequences, including but not limited to, non-coding 5' and 3' sequences, such as the transcribed, non-translated sequences that play a role in transcription, mRNA processing, including splicing and polyadenylation signals (for example - ribosome binding and stability of mRNA); an additional coding sequence that codes for additional amino acids, such as those that provide additional functionalities. Thus, the sequence encoding an antibody can be fused to a marker sequence, such as a sequence encoding a peptide that facilitates purification of the fused antibody comprising an antibody fragment or portion.

#### **Polynucleotides Which Selectively Hybridize to a Polynucleotide as Described Herein**

The present invention provides isolated nucleic acids that hybridize under selective hybridization conditions to a polynucleotide disclosed herein. Thus, the polynucleotides of this embodiment can be used for isolating, detecting, and/or quantifying nucleic acids comprising such polynucleotides. For example, polynucleotides of the present invention can be used to identify, isolate,

or amplify partial or full-length clones in a deposited library. In some embodiments, the polynucleotides are genomic or cDNA sequences isolated, or otherwise complementary to, a cDNA from a human or mammalian nucleic acid library.

- Preferably, the cDNA library comprises at least 80% full-length sequences, preferably at least 5 85% or 90% full-length sequences, and more preferably at least 95% full-length sequences. The cDNA libraries can be normalized to increase the representation of rare sequences. Low or moderate stringency hybridization conditions are typically, but not exclusively, employed with sequences having a reduced sequence identity relative to complementary sequences. Moderate and high stringency conditions can optionally be employed for sequences of greater identity. Low stringency conditions 10 allow selective hybridization of sequences having about 70% sequence identity and can be employed to identify orthologous or paralogous sequences.

Optionally, polynucleotides of this invention will encode at least a portion of an antibody encoded by the polynucleotides described herein. The polynucleotides of this invention embrace nucleic acid sequences that can be employed for selective hybridization to a polynucleotide encoding an 15 antibody of the present invention. See, e.g., Ausubel, *supra*; Colligan, *supra*, each entirely incorporated herein by reference.

#### **Construction of Nucleic Acids**

The isolated nucleic acids of the present invention can be made using (a) recombinant methods, (b) synthetic techniques, (c) purification techniques, or combinations thereof, as well-known in the art.

20 The nucleic acids can conveniently comprise sequences in addition to a polynucleotide of the present invention. For example, a multi-cloning site comprising one or more endonuclease restriction sites can be inserted into the nucleic acid to aid in isolation of the polynucleotide. Also, translatable sequences can be inserted to aid in the isolation of the translated polynucleotide of the present invention. For example, a hexa-histidine marker sequence provides a convenient means to purify the proteins of 25 the present invention. The nucleic acid of the present invention - excluding the coding sequence - is optionally a vector, adapter, or linker for cloning and/or expression of a polynucleotide of the present invention.

Additional sequences can be added to such cloning and/or expression sequences to optimize their function in cloning and/or expression, to aid in isolation of the polynucleotide, or to improve the 30 introduction of the polynucleotide into a cell. Use of cloning vectors, expression vectors, adapters, and linkers is well known in the art. (See, e.g., Ausubel, *supra*; or Sambrook, *supra*)

#### **Recombinant Methods for Constructing Nucleic Acids**

- The isolated nucleic acid compositions of this invention, such as RNA, cDNA, genomic DNA, or any combination thereof, can be obtained from biological sources using any number of cloning methodologies known to those of skill in the art. In some embodiments, oligonucleotide probes that selectively hybridize, under stringent conditions, to the polynucleotides of the present invention are used
- 5 to identify the desired sequence in a cDNA or genomic DNA library. The isolation of RNA, and construction of cDNA and genomic libraries, is well known to those of ordinary skill in the art. (See, e.g., Ausubel, *supra*; or Sambrook, *supra*)

#### Nucleic Acid Screening and Isolation Methods

A cDNA or genomic library can be screened using a probe based upon the sequence of a

10 polynucleotide of the present invention, such as those disclosed herein. Probes can be used to hybridize with genomic DNA or cDNA sequences to isolate homologous genes in the same or different organisms. Those of skill in the art will appreciate that various degrees of stringency of hybridization can be employed in the assay; and either the hybridization or the wash medium can be stringent. As the conditions for hybridization become more stringent, there must be a greater degree of complementarity

15 between the probe and the target for duplex formation to occur. The degree of stringency can be controlled by one or more of temperature, ionic strength, pH and the presence of a partially denaturing solvent such as formamide. For example, the stringency of hybridization is conveniently varied by changing the polarity of the reactant solution through, for example, manipulation of the concentration of formamide within the range of 0% to 50%. The degree of complementarity (sequence identity) required

20 for detectable binding will vary in accordance with the stringency of the hybridization medium and/or wash medium. The degree of complementarity will optimally be 100%, or 70-100%, or any range or value therein. However, it should be understood that minor sequence variations in the probes and primers can be compensated for by reducing the stringency of the hybridization and/or wash medium.

Methods of amplification of RNA or DNA are well known in the art and can be used

25 according to the present invention without undue experimentation, based on the teaching and guidance presented herein.

Known methods of DNA or RNA amplification include, but are not limited to, polymerase chain reaction (PCR) and related amplification processes (see, e.g., U.S. Patent Nos. 4,683,195, 4,683,202, 4,800,159, 4,965,188, to Mullis, et al.; 4,795,699 and 4,921,794 to Tabor, et al; 5,142,033 to Innis; 5,122,464 to Wilson, et al.; 5,091,310 to Innis; 5,066,584 to Gyllensten, et al; 4,889,818 to Gelfand, et al; 4,994,370 to Silver, et al; 4,766,067 to Biswas; 4,656,134 to Ringold) and RNA mediated amplification that uses anti-sense RNA to the target sequence as a template for double-stranded DNA synthesis (U.S. Patent No. 5,130,238 to Malek, et al, with the tradename NASBA), the

entire contents of which references are incorporated herein by reference. (See, e.g., Ausubel, *supra*; or Sambrook, *supra*.)

For instance, polymerase chain reaction (PCR) technology can be used to amplify the sequences of polynucleotides of the present invention and related genes directly from genomic DNA or cDNA libraries. PCR and other in vitro amplification methods can also be useful, for example, to clone nucleic acid sequences that code for proteins to be expressed, to make nucleic acids to use as probes for detecting the presence of the desired mRNA in samples, for nucleic acid sequencing, or for other purposes. Examples of techniques sufficient to direct persons of skill through in vitro amplification methods are found in Berger, *supra*, Sambrook, *supra*, and Ausubel, *supra*, as well as Mullis, et al., U.S. Patent No. 4,683,202 (1987); and Innis, et al., PCR Protocols A Guide to Methods and Applications, Eds., Academic Press Inc., San Diego, CA (1990). Commercially available kits for genomic PCR amplification are known in the art. See, e.g., Advantage-GC Genomic PCR Kit (Clontech). Additionally, e.g., the T4 gene 32 protein (Boehringer Mannheim) can be used to improve yield of long PCR products.

#### 15 Synthetic Methods for Constructing Nucleic Acids

The isolated nucleic acids of the present invention can also be prepared by direct chemical synthesis by known methods (see, e.g., Ausubel, et al., *supra*). Chemical synthesis generally produces a single-stranded oligonucleotide, which can be converted into double-stranded DNA by hybridization with a complementary sequence, or by polymerization with a DNA polymerase using the single strand as a template. One of skill in the art will recognize that while chemical synthesis of DNA can be limited to sequences of about 100 or more bases, longer sequences can be obtained by the ligation of shorter sequences.

#### Recombinant Expression Cassettes

The present invention further provides recombinant expression cassettes comprising a nucleic acid of the present invention. A nucleic acid sequence of the present invention, for example a cDNA or a genomic sequence encoding an antibody of the present invention, can be used to construct a recombinant expression cassette that can be introduced into at least one desired host cell. A recombinant expression cassette will typically comprise a polynucleotide of the present invention operably linked to transcriptional initiation regulatory sequences that will direct the transcription of the polynucleotide in the intended host cell. Both heterologous and non-heterologous (i.e., endogenous) promoters can be employed to direct expression of the nucleic acids of the present invention.

In some embodiments, isolated nucleic acids that serve as promoter, enhancer, or other elements can be introduced in the appropriate position (upstream, downstream or in intron) of a non-heterologous

form of a polynucleotide of the present invention so as to up or down regulate expression of a polynucleotide of the present invention. For example, endogenous promoters can be altered *in vivo* or *in vitro* by mutation, deletion and/or substitution.

#### Vectors And Host Cells

5        The present invention also relates to vectors that include isolated nucleic acid molecules of the present invention, host cells that are genetically engineered with the recombinant vectors, and the production of at least one anti-TNF antibody by recombinant techniques, as is well known in the art. See, e.g., Sambrook, et al., *supra*; Ausubel, et al., *supra*, each entirely incorporated herein by reference.

10      The polynucleotides can optionally be joined to a vector containing a selectable marker for propagation in a host. Generally, a plasmid vector is introduced in a precipitate, such as a calcium phosphate precipitate, or in a complex with a charged lipid. If the vector is a virus, it can be packaged *in vitro* using an appropriate packaging cell line and then transduced into host cells.

15      The DNA insert should be operatively linked to an appropriate promoter. The expression constructs will further contain sites for transcription initiation, termination and, in the transcribed region, a ribosome binding site for translation. The coding portion of the mature transcripts expressed by the constructs will preferably include a translation initiating at the beginning and a termination codon (e.g., UAA, UGA or UAG) appropriately positioned at the end of the mRNA to be translated, with UAA and UAG preferred for mammalian or eukaryotic cell expression.

20      Expression vectors will preferably but optionally include at least one selectable marker. Such markers include, e.g., but not limited to, methotrexate (MTX), dihydrofolate reductase (DHFR, US Pat.Nos. 4,399,216; 4,634,665; 4,656,134; 4,956,288; 5,149,636; 5,179,017, ampicillin, neomycin (G418), mycophenolic acid, or glutamine synthetase (GS, US Pat.Nos. 5,122,464; 5,770,359; 5,827,739) resistance for eukaryotic cell culture, and tetracycline or ampicillin resistance genes for 25 culturing in *E. coli* and other bacteria or prokaryotics (the above patents are entirely incorporated hereby by reference). Appropriate culture mediums and conditions for the above-described host cells are known in the art. Suitable vectors will be readily apparent to the skilled artisan. Introduction of a vector construct into a host cell can be effected by calcium phosphate transfection, DEAE-dextran mediated transfection, cationic lipid-mediated transfection, electroporation, transduction, infection or 30 other known methods. Such methods are described in the art, such as Sambrook, *supra*, Chapters 1-4 and 16-18; Ausubel, *supra*, Chapters 1, 9, 13, 15, 16.

At least one antibody of the present invention can be expressed in a modified form, such as a fusion protein, and can include not only secretion signals, but also additional heterologous functional

regions. For instance, a region of additional amino acids, particularly charged amino acids, can be added to the N-terminus of an antibody to improve stability and persistence in the host cell, during purification, or during subsequent handling and storage. Also, peptide moieties can be added to an antibody of the present invention to facilitate purification. Such regions can be removed prior to final preparation of an antibody or at least one fragment thereof. Such methods are described in many standard laboratory manuals, such as Sambrook, *supra*, Chapters 17.29-17.42 and 18.1-18.74; Ausubel, *supra*, Chapters 16, 17 and 18.

Those of ordinary skill in the art are knowledgeable in the numerous expression systems available for expression of a nucleic acid encoding a protein of the present invention.

10 Alternatively, nucleic acids of the present invention can be expressed in a host cell by turning on (by manipulation) in a host cell that contains endogenous DNA encoding an antibody of the present invention. Such methods are well known in the art, e.g., as described in US patent Nos. 5,580,734, 5,641,670, 5,733,746, and 5,733,761, entirely incorporated herein by reference.

15 Illustrative of cell cultures useful for the production of the antibodies, specified portions or variants thereof, are mammalian cells. Mammalian cell systems often will be in the form of monolayers of cells although mammalian cell suspensions or bioreactors can also be used. A number of suitable host cell lines capable of expressing intact glycosylated proteins have been developed in the art, and include the COS-1 (e.g., ATCC CRL 1650), COS-7 (e.g., ATCC CRL-1651), HEK293, BHK21 (e.g., ATCC CRL-10), CHO (e.g., ATCC CRL 1610) and BSC-1 (e.g., ATCC CRL-26) cell lines, Cos-7 20 cells, CHO cells, hep G2 cells, P3X63Ag8.653, SP2/0-Ag14, 293 cells, HeLa cells and the like, which are readily available from, for example, American Type Culture Collection, Manassas, Va (www.atcc.org). Preferred host cells include cells of lymphoid origin such as myeloma and lymphoma cells. Particularly preferred host cells are P3X63Ag8.653 cells (ATCC Accession Number CRL-1580) and SP2/0-Ag14 cells (ATCC Accession Number CRL-1851). In a particularly 25 preferred embodiment, the recombinant cell is a P3X63Ab8.653 or a SP2/0-Ag14 cell.

Expression vectors for these cells can include one or more of the following expression control sequences, such as, but not limited to an origin of replication; a promoter (e.g., late or early SV40 promoters, the CMV promoter (US Pat.Nos. 5,168,062; 5,385,839), an HSV tk promoter, a pgk (phosphoglycerate kinase) promoter, an EF-1 alpha promoter (US Pat.No. 5,266,491), at least one 30 human immunoglobulin promoter; an enhancer, and/or processing information sites, such as ribosome binding sites, RNA splice sites, polyadenylation sites (e.g., an SV40 large T Ag poly A addition site), and transcriptional terminator sequences. See, e.g., Ausubel et al., *supra*; Sambrook, et al., *supra*. Other cells useful for production of nucleic acids or proteins of the present invention are known and/or

available, for instance, from the American Type Culture Collection Catalogue of Cell Lines and Hybridomas ([www.atcc.org](http://www.atcc.org)) or other known or commercial sources.

When eukaryotic host cells are employed, polyadenylation or transcription terminator sequences are typically incorporated into the vector. An example of a terminator sequence is the polyadenylation sequence from the bovine growth hormone gene. Sequences for accurate splicing of the transcript can also be included. An example of a splicing sequence is the VP1 intron from SV40 (Sprague, et al., J. Virol. 45:773-781 (1983)). Additionally, gene sequences to control replication in the host cell can be incorporated into the vector, as known in the art.

#### 10    Purification of an Antibody

An anti-TNF antibody can be recovered and purified from recombinant cell cultures by well-known methods including, but not limited to, protein A purification, ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. High performance liquid chromatography ("HPLC") can also be employed for purification. See, e.g., Colligan, Current Protocols in Immunology, or Current Protocols in Protein Science, John Wiley & Sons, NY, NY, (1997-2001), e.g., Chapters 1, 4, 6, 8, 9, 10, each entirely incorporated herein by reference.

Antibodies of the present invention include naturally purified products, products of chemical synthetic procedures, and products produced by recombinant techniques from a eukaryotic host, including, for example, yeast, higher plant, insect and mammalian cells. Depending upon the host employed in a recombinant production procedure, the antibody of the present invention can be glycosylated or can be non-glycosylated, with glycosylated preferred. Such methods are described in many standard laboratory manuals, such as Sambrook, *supra*, Sections 17.37-17.42; Ausubel, *supra*, Chapters 10, 12, 13, 16, 18 and 20, Colligan, *Protein Science*, *supra*, Chapters 12-14, all entirely incorporated herein by reference.

#### Anti-TNF Antibodies

The isolated antibodies of the present invention comprise an antibody amino acid sequences disclosed herein encoded by any suitable polynucleotide, or any isolated or prepared antibody. Preferably, the human antibody or antigen-binding fragment binds human TNF and, thereby partially or substantially neutralizes at least one biological activity of the protein. An antibody, or specified portion or variant thereof, that partially or preferably substantially neutralizes at least one biological activity of

at least one TNF protein or fragment can bind the protein or fragment and thereby inhibit activity mediated through the binding of TNF to the TNF receptor or through other TNF-dependent or mediated mechanisms. As used herein, the term "neutralizing antibody" refers to an antibody that can inhibit an TNF-dependent activity by about 20-120%, preferably by at least about 10, 20, 30, 40, 50, 55, 60, 65,  
5 70, 75, 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100% or more depending on the assay. The capacity of an anti-TNF antibody to inhibit an TNF-dependent activity is preferably assessed by at least one suitable TNF protein or receptor assay, as described herein and/or as known in the art. A human antibody of the invention can be of any class (IgG, IgA, IgM, IgE, IgD, etc.) or isotype and can comprise a kappa or lambda light chain. In one embodiment, the human antibody comprises an IgG heavy chain  
10 or defined fragment, for example, at least one of isotypes, IgG1, IgG2, IgG3 or IgG4. Antibodies of this type can be prepared by employing a transgenic mouse or other transgenic non-human mammal comprising at least one human light chain (e.g., IgG, IgA and IgM (e.g.,  $\gamma$ 1,  $\gamma$ 2,  $\gamma$ 3,  $\gamma$ 4) transgenes as described herein and/or as known in the art. In another embodiment, the anti-human TNF human antibody comprises an IgG1 heavy chain and a IgG1 light chain.  
15 At least one antibody of the invention binds at least one specified epitope specific to at least one TNF protein, subunit, fragment, portion or any combination thereof. The at least one epitope can comprise at least one antibody binding region that comprises at least one portion of the protein, which epitope is preferably comprised of at least one extracellular, soluble, hydrophilic, external or cytoplasmic portion of the protein. The at least one specified epitope can comprise any combination  
20 of at least one amino acid sequence of at least 1-3 amino acids to the entire specified portion of contiguous amino acids of the SEQ ID NO:17.

Generally, the human antibody or antigen-binding fragment of the present invention will comprise an antigen-binding region that comprises at least one human complementarity determining region (CDR1, CDR2 and CDR3) or variant of at least one heavy chain variable region and at least  
25 one human complementarity determining region (CDR1, CDR2 and CDR3) or variant of at least one light chain variable region. As a non-limiting example, the antibody or antigen-binding portion or variant can comprise at least one of the heavy chain CDR3 having the amino acid sequence of SEQ ID NO:3 or 11, and/or a light chain CDR3 having the amino acid sequence of SEQ ID NO:6 or 14. In a particular embodiment, the antibody or antigen-binding fragment can have an antigen-binding  
30 region that comprises at least a portion of at least one heavy chain CDR (i.e., CDR1, CDR2 and/or CDR3) having the amino acid sequence of the corresponding CDRs 1, 2 and/or 3 (e.g., SEQ ID NOS:1, 2, 3, 9, 10, and/or 11). In another particular embodiment, the antibody or antigen-binding portion or variant can have an antigen-binding region that comprises at least a portion of at least one

light chain CDR (i.e., CDR1, CDR2 and/or CDR3) having the amino acid sequence of the corresponding CDRs 1, 2 and/or 3 (e.g., SEQ ID NOS: 4, 5, 6, 12,13, and/or 14). In a preferred embodiment the three heavy chain CDRs and the three light chain CDRs of the antibody or antigen-binding fragment have the amino acid sequence of the corresponding CDR of at least one of mAb 5 9C1A, 9E7, as described herein. Such antibodies can be prepared by chemically joining together the various portions (e.g., CDRs, framework) of the antibody using conventional techniques, by preparing and expressing a (i.e., one or more) nucleic acid molecule that encodes the antibody using conventional techniques of recombinant DNA technology or by using any other suitable method.

The anti-TNF antibody can comprise at least one of a heavy or light chain variable region 10 having a defined amino acid sequence. For example, in a preferred embodiment, the anti-TNF antibody comprises at least one of at least one heavy chain variable region, optionally having the amino acid sequence of SEQ ID NOS:7 or 15 and/or at least one light chain variable region, optionally having the amino acid sequence of SEQ ID NOS:8 or 16. Antibodies that bind to human TNF and that comprise a defined heavy or light chain variable region can be prepared using suitable 15 methods, such as phage display (Katsube, Y., et al., *Int J Mol. Med.*, 1(5):863-868 (1998)) or methods that employ transgenic animals, as known in the art and/or as described herein. For example, a transgenic mouse, comprising a functionally rearranged human immunoglobulin heavy chain transgene and a transgene comprising DNA from a human immunoglobulin light chain locus that can undergo functional rearrangement, can be immunized with human TNF or a fragment thereof to elicit 20 the production of antibodies. If desired, the antibody producing cells can be isolated and hybridomas or other immortalized antibody-producing cells can be prepared as described herein and/or as known in the art. Alternatively, the antibody, specified portion or variant can be expressed using the encoding nucleic acid or portion thereof in a suitable host cell.

The invention also relates to antibodies, antigen-binding fragments, immunoglobulin chains 25 and CDRs comprising amino acids in a sequence that is substantially the same as an amino acid sequence described herein. Preferably, such antibodies or antigen-binding fragments and antibodies comprising such chains or CDRs can bind human TNF with high affinity (e.g.,  $K_D$  less than or equal to about  $10^{-9}$  M). Amino acid sequences that are substantially the same as the sequences described herein include sequences comprising conservative amino acid substitutions, as well as amino acid 30 deletions and/or insertions. A conservative amino acid substitution refers to the replacement of a first amino acid by a second amino acid that has chemical and/or physical properties (e.g, charge, structure, polarity, hydrophobicity/ hydrophilicity) that are similar to those of the first amino acid. Conservative substitutions include replacement of one amino acid by another within the following

groups: lysine (K), arginine (R) and histidine (H); aspartate (D) and glutamate (E); asparagine (N), glutamine (Q), serine (S), threonine (T), tyrosine (Y), K, R, H, D and E; alanine (A), valine (V), leucine (L), isoleucine (I), proline (P), phenylalanine (F), tryptophan (W), methionine (M), cysteine (C) and glycine (G); F, W and Y; C, S and T.

## 5 Amino Acid Codes

The amino acids that make up anti-TNF antibodies of the present invention are often abbreviated. The amino acid designations can be indicated by designating the amino acid by its single letter code, its three letter code, name, or three nucleotide codon(s) as is well understood in the art (see Alberts, B., et al., Molecular Biology of The Cell, Third Ed., Garland Publishing, Inc., New

10 York, 1994):

SINGLE LETTER CODE	THREE LETTER CODE	NAME	THREE NUCLEOTIDE CODON(S)
A	Ala	Alanine	GCA, GCC, GCG, GCU
C	Cys	Cysteine	UGC, UGU
D	Asp	Aspartic acid	GAC, GAU
E	Glu	Glutamic acid	GAA, GAG
F	Phe	Phenylalanine	UUC, UUU
G	Gly	Glycine	GGA, GGC, GGG, GGU
H	His	Histidine	CAC, CAU
I	Ile	Isoleucine	AUA, AUC, AUU
K	Lys	Lysine	AAA, AAG
L	Leu	Leucine	UUA, UUG, CUA, CUC, CUG, CUU
M	Met	Methionine	AUG
N	Asn	Asparagine	AAC, AAU
P	Pro	Proline	CCA, CCC, CCG, CCU
Q	Gln	Glutamine	CAA, CAG
R	Arg	Arginine	AGA, AGG, CGA, CGC, CGG, CGU
S	Ser	Serine	AGC, AGU, UCA, UCC, UCG, UCU
T	Thr	Threonine	ACA, ACC, ACG, ACU
V	Val	Valine	GUU, GUC, GUG, GUU
W	Trp	Tryptophan	UGG
Y	Tyr	Tyrosine	UAC, UAU

An anti-TNF antibody of the present invention can include one or more amino acid substitutions, deletions or additions, either from natural mutations or human manipulation, as  
15 specified herein.

Of course, the number of amino acid substitutions a skilled artisan would make depends on many factors, including those described above. Generally speaking, the number of amino acid substitutions, insertions or deletions for any given anti-TNF antibody, fragment or variant will not be more than 40, 30, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1, such as 1-30 or any 5 range or value therein, as specified herein.

Amino acids in an anti-TNF antibody of the present invention that are essential for function can be identified by methods known in the art, such as site-directed mutagenesis or alanine-scanning mutagenesis (e.g., Ausubel, *supra*, Chapters 8, 15; Cunningham and Wells, *Science* 244:1081-1085 (1989)). The latter procedure introduces single alanine mutations at every residue in the molecule. 10 The resulting mutant molecules are then tested for biological activity, such as, but not limited to at least one TNF neutralizing activity. Sites that are critical for antibody binding can also be identified by structural analysis such as crystallization, nuclear magnetic resonance or photoaffinity labeling (Smith, et al., *J. Mol. Biol.* 224:899-904 (1992) and de Vos, et al., *Science* 255:306-312 (1992)).

Anti-TNF antibodies of the present invention can include, but are not limited to, at least one portion, sequence or combination selected from 5 to all of the contiguous amino acids of at least one of SEQ ID NOS:1, 2, 3, 4, 5, 6 and/or 9, 10, 11, 12, 13 and 14. 15

A(n) anti-TNF antibody can further optionally comprise a polypeptide of at least one of 70-100% of the contiguous amino acids of at least one of SEQ ID NOS:7, 8, 15, 16.

In one embodiment, the amino acid sequence of an immunoglobulin chain, or portion thereof 20 (e.g., variable region, CDR) has about 70-100% identity (e.g., 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100 or any range or value therein) to the amino acid sequence of the corresponding chain of at least one of SEQ ID NOS:7, 8, 15, 16. For example, the amino acid sequence of a light chain variable region can be compared with the sequence of SEQ ID NO:8 or 16, or the amino acid sequence of a heavy chain CDR3 can be 25 compared with SEQ ID NO:7 or 15. Preferably, 70-100% amino acid identity (i.e., 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100 or any range or value therein) is determined using a suitable computer algorithm, as known in the art.

Exemplary heavy chain and light chain variable regions sequences are provided in SEQ ID NOS: 7, 8, 15, 16. The antibodies of the present invention, or specified variants thereof, can comprise 30 any number of contiguous amino acid residues from an antibody of the present invention, wherein that number is selected from the group of integers consisting of from 10-100% of the number of contiguous residues in an anti-TNF antibody. Optionally, this subsequence of contiguous amino acids is at least about 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220,

230, 240, 250 or more amino acids in length, or any range or value therein. Further, the number of such subsequences can be any integer selected from the group consisting of from 1 to 20, such as at least 2, 3, 4, or 5.

As those of skill will appreciate, the present invention includes at least one biologically active antibody of the present invention. Biologically active antibodies have a specific activity at least 20%, 30%, or 40%, and preferably at least 50%, 60%, or 70%, and most preferably at least 80%, 90%, or 95%-1000% of that of the native (non-synthetic), endogenous or related and known antibody. Methods of assaying and quantifying measures of enzymatic activity and substrate specificity, are well known to those of skill in the art.

In another aspect, the invention relates to human antibodies and antigen-binding fragments, as described herein, which are modified by the covalent attachment of an organic moiety. Such modification can produce an antibody or antigen-binding fragment with improved pharmacokinetic properties (e.g., increased *in vivo* serum half-life). The organic moiety can be a linear or branched hydrophilic polymeric group, fatty acid group, or fatty acid ester group. In particular embodiments, the hydrophilic polymeric group can have a molecular weight of about 800 to about 120,000 Daltons and can be a polyalkane glycol (e.g., polyethylene glycol (PEG), polypropylene glycol (PPG)), carbohydrate polymer, amino acid polymer or polyvinyl pyrrolidone, and the fatty acid or fatty acid ester group can comprise from about eight to about forty carbon atoms.

The modified antibodies and antigen-binding fragments of the invention can comprise one or more organic moieties that are covalently bonded, directly or indirectly, to the antibody. Each organic moiety that is bonded to an antibody or antigen-binding fragment of the invention can independently be a hydrophilic polymeric group, a fatty acid group or a fatty acid ester group. As used herein, the term "fatty acid" encompasses mono-carboxylic acids and di-carboxylic acids. A "hydrophilic polymeric group," as the term is used herein, refers to an organic polymer that is more soluble in water than in octane. For example, polylysine is more soluble in water than in octane. Thus, an antibody modified by the covalent attachment of polylysine is encompassed by the invention. Hydrophilic polymers suitable for modifying antibodies of the invention can be linear or branched and include, for example, polyalkane glycals (e.g., PEG, monomethoxy-polyethylene glycol (mPEG), PPG and the like), carbohydrates (e.g., dextran, cellulose, oligosaccharides, polysaccharides and the like), polymers of hydrophilic amino acids (e.g., polylysine, polyarginine, polyaspartate and the like), polyalkane oxides (e.g., polyethylene oxide, polypropylene oxide and the like) and polyvinyl pyrrolidone. Preferably, the hydrophilic polymer that modifies the antibody of the invention has a molecular weight of about 800 to about 150,000 Daltons as a separate molecular entity. For

example PEG<sub>5000</sub> and PEG<sub>20,000</sub>, wherein the subscript is the average molecular weight of the polymer in Daltons, can be used. The hydrophilic polymeric group can be substituted with one to about six alkyl, fatty acid or fatty acid ester groups. Hydrophilic polymers that are substituted with a fatty acid or fatty acid ester group can be prepared by employing suitable methods. For example, a polymer 5 comprising an amine group can be coupled to a carboxylate of the fatty acid or fatty acid ester, and an activated carboxylate (e.g., activated with N, N-carbonyl diimidazole) on a fatty acid or fatty acid ester can be coupled to a hydroxyl group on a polymer.

Fatty acids and fatty acid esters suitable for modifying antibodies of the invention can be saturated or can contain one or more units of unsaturation. Fatty acids that are suitable for modifying 10 antibodies of the invention include, for example, n-dodecanoate (C<sub>12</sub>, laurate), n-tetradecanoate (C<sub>14</sub>, myristate), n-octadecanoate (C<sub>18</sub>, stearate), n-eicosanoate (C<sub>20</sub>, arachidate), n-docosanoate (C<sub>22</sub>, behenate), n-triacontanoate (C<sub>30</sub>), n-tetracontanoate (C<sub>40</sub>), *cis*-Δ9-octadecanoate (C<sub>18</sub>, oleate), all *cis*-Δ5,8,11,14-eicosatetraenoate (C<sub>20</sub>, arachidonate), octanedioic acid, tetradecanedioic acid, 15 octadecanedioic acid, docosanedioic acid, and the like. Suitable fatty acid esters include mono-esters of dicarboxylic acids that comprise a linear or branched lower alkyl group. The lower alkyl group can comprise from one to about twelve, preferably one to about six, carbon atoms.

The modified human antibodies and antigen-binding fragments can be prepared using suitable methods, such as by reaction with one or more modifying agents. A "modifying agent" as 20 the term is used herein, refers to a suitable organic group (e.g., hydrophilic polymer, a fatty acid, a fatty acid ester) that comprises an activating group. An "activating group" is a chemical moiety or functional group that can, under appropriate conditions, react with a second chemical group thereby forming a covalent bond between the modifying agent and the second chemical group. For example, amine-reactive activating groups include electrophilic groups such as tosylate, mesylate, halo (chloro, bromo, fluoro, iodo), N-hydroxysuccinimidyl esters (NHS), and the like. Activating groups that can react with thiols include, for example, maleimide, iodoacetyl, acryloyl, pyridyl disulfides, 5-thiol-2-nitrobenzoic acid thiol (TNB-thiol), and the like. An aldehyde functional group can be coupled to 25 amine- or hydrazide-containing molecules, and an azide group can react with a trivalent phosphorous group to form phosphoramidate or phosphorimide linkages. Suitable methods to introduce activating groups into molecules are known in the art (see for example, Hermanson, G. T., *Bioconjugate Techniques*, Academic Press: San Diego, CA (1996)). An activating group can be bonded directly to the organic group (e.g., hydrophilic polymer, fatty acid, fatty acid ester), or through a linker moiety, 30 for example a divalent C<sub>1</sub>-C<sub>12</sub> group wherein one or more carbon atoms can be replaced by a heteroatom such as oxygen, nitrogen or sulfur. Suitable linker moieties include, for example,

tetraethylene glycol, -(CH<sub>2</sub>)<sub>3</sub>- , -NH-(CH<sub>2</sub>)<sub>6</sub>-NH-, -(CH<sub>2</sub>)<sub>2</sub>-NH- and -CH<sub>2</sub>-O-CH<sub>2</sub>-CH<sub>2</sub>-O-CH<sub>2</sub>-CH<sub>2</sub>-O-CH-NH-. Modifying agents that comprise a linker moiety can be produced, for example, by reacting a mono-Boc-alkyldiamine (e.g., mono-Boc-ethylenediamine, mono-Boc-diaminohexane) with a fatty acid in the presence of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) to form an amide bond between the free amine and the fatty acid carboxylate. The Boc protecting group can be removed from the product by treatment with trifluoroacetic acid (TFA) to expose a primary amine that can be coupled to another carboxylate as described, or can be reacted with maleic anhydride and the resulting product cyclized to produce an activated maleimido derivative of the fatty acid. (See, for example, Thompson, *et al.*, WO 92/16221 the entire teachings of which are incorporated herein by reference.)

The modified antibodies of the invention can be produced by reacting a human antibody or antigen-binding fragment with a modifying agent. For example, the organic moieties can be bonded to the antibody in a non-site specific manner by employing an amine-reactive modifying agent, for example, an NHS ester of PEG. Modified human antibodies or antigen-binding fragments can also be prepared by reducing disulfide bonds (e.g., intra-chain disulfide bonds) of an antibody or antigen-binding fragment. The reduced antibody or antigen-binding fragment can then be reacted with a thiol-reactive modifying agent to produce the modified antibody of the invention. Modified human antibodies and antigen-binding fragments comprising an organic moiety that is bonded to specific sites of an antibody of the present invention can be prepared using suitable methods, such as reverse proteolysis (Fisch *et al.*, *Bioconjugate Chem.*, 3:147-153 (1992); Werlen *et al.*, *Bioconjugate Chem.*, 5:411-417 (1994); Kumaran *et al.*, *Protein Sci.* 6(10):2233-2241 (1997); Itoh *et al.*, *Bioorg. Chem.*, 24(1): 59-68 (1996); Capellas *et al.*, *Biotechnol. Bioeng.*, 56(4):456-463 (1997)), and the methods described in Hermanson, G. T., *Bioconjugate Techniques*, Academic Press: San Diego, CA (1996).

#### ANTI-IDIOTYPE ANTIBODIES TO ANTI-TNF ANTIBODY COMPOSITIONS

In addition to monoclonal or chimeric anti-TNF antibodies, the present invention is also directed to an anti-idiotypic (anti-Id) antibody specific for such antibodies of the invention. An anti-Id antibody is an antibody which recognizes unique determinants generally associated with the antigen-binding region of another antibody. The anti-Id can be prepared by immunizing an animal of the same species and genetic type (e.g. mouse strain) as the source of the Id antibody with the antibody or a CDR containing region thereof. The immunized animal will recognize and respond to the idiotypic determinants of the immunizing antibody and produce an anti-Id antibody. The anti-Id antibody may also be used as an "immunogen" to induce an immune response in yet another animal, producing a so-called anti-anti-Id antibody.

The present invention also provides at least one anti-TNF antibody composition comprising at least one, at least two, at least three, at least four, at least five, at least six or more anti-TNF antibodies thereof, as described herein and/or as known in the art that are provided in a non-naturally occurring composition, mixture or form. Such compositions comprise non-naturally occurring

5 compositions comprising at least one or two full length, C- and/or N-terminally deleted variants, domains, fragments, or specified variants, of the anti-TNF antibody amino acid sequence selected from the group consisting of 70-100% of the contiguous amino acids of SEQ ID NOS:1, 2, 3, 4, 5, 6, 7, 8, 11, 12, 13, 14, 15, 16, or specified fragments, domains or variants thereof. Preferred anti-TNF antibody compositions include at least one or two full length, fragments, domains or variants as at

10 least one CDR or LBP containing portions of the anti-TNF antibody sequence of 70-100% of SEQ ID NOS:1, 2, 3, 4, 5, 6, 9, 10, 11, 12, 13, 14, or specified fragments, domains or variants thereof. Further preferred compositions comprise 40-99% of at least one of 70-100% of SEQ ID NOS:1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or specified fragments, domains or variants thereof. Such

15 composition percentages are by weight, volume, concentration, molarity, or molality as liquid or dry solutions, mixtures, suspension, emulsions, particles, powder, or colloids, as known in the art or as described herein.

Anti-TNF antibody compositions of the present invention can further comprise at least one of any suitable and effective amount of a composition or pharmaceutical composition comprising at least one anti-TNF antibody to a cell, tissue, organ, animal or patient in need of such modulation, treatment or therapy, optionally further comprising at least one selected from at least one TNF antagonist (e.g., but not limited to a TNF antibody or fragment, a soluble TNF receptor or fragment, fusion proteins thereof, or a small molecule TNF antagonist), an antirheumatic (e.g., methotrexate, auranofin, aurothioglucose, azathioprine, etanercept, gold sodium thiomalate, hydroxychloroquine sulfate, leflunomide, sulfasalazine), a muscle relaxant, a narcotic, a non-steroid anti-inflammatory drug (NSAID), an analgesic, an anesthetic, a sedative, a local anesthetic, a neuromuscular blocker, an antimicrobial (e.g., aminoglycoside, an antifungal, an antiparasitic, an antiviral, a carbapenem, cephalosporin, a fluorquinolone, a macrolide, a penicillin, a sulfonamide, a tetracycline, another antimicrobial), an antipsoriatic, a corticosteroid, an anabolic steroid, a diabetes related agent, a mineral, a nutritional, a thyroid agent, a vitamin, a calcium related hormone, an antidiarrheal, an antitussive, an antiemetic, an antiulcer, a laxative, an anticoagulant, an erythropoietin (e.g., epoetin alpha), a filgrastim (e.g., G-CSF, Neupogen), a sargramostim (GM-CSF, Leukine), an immunization, an immunoglobulin, an immunosuppressive (e.g., basiliximab, cyclosporine, daclizumab), a growth hormone, a hormone replacement drug, an estrogen receptor modulator, a mydriatic, a cycloplegic, an

alkylating agent, an antimetabolite, a mitotic inhibitor, a radiopharmaceutical, an antidepressant, antimanic agent, an antipsychotic, an anxiolytic, a hypnotic, a sympathomimetic, a stimulant, donepezil, tacrine, an asthma medication, a beta agonist, an inhaled steroid, a leukotriene inhibitor, a methylxanthine, a cromolyn, an epinephrine or analog, dornase alpha (Pulmozyme), a cytokine or a cytokine antagonist. Non-limiting examples of such cytokines include, but are not limited to, any of IL-1 to IL-23. Suitable dosages are well known in the art. See, e.g., Wells et al., eds., *Pharmacotherapy Handbook*, 2<sup>nd</sup> Edition, Appleton and Lange, Stamford, CT (2000); PDR *Pharmacopoeia*, Tarascon Pocket Pharmacopoeia 2000, Deluxe Edition, Tarascon Publishing, Loma Linda, CA (2000), each of which references are entirely incorporated herein by reference.

Such anti-cancer or anti-infectives can also include toxin molecules that are associated, bound, co-formulated or co-administered with at least one antibody of the present invention. The toxin can optionally act to selectively kill the pathologic cell or tissue. The pathologic cell can be a cancer or other cell. Such toxins can be, but are not limited to, purified or recombinant toxin or toxin fragment comprising at least one functional cytotoxic domain of toxin, e.g., selected from at least one of ricin, diphtheria toxin, a venom toxin, or a bacterial toxin. The term toxin also includes both endotoxins and exotoxins produced by any naturally occurring, mutant or recombinant bacteria or viruses which may cause any pathological condition in humans and other mammals, including toxin shock, which can result in death. Such toxins may include, but are not limited to, enterotoxigenic *E. coli* heat-labile enterotoxin (LT), heat-stable enterotoxin (ST), *Shigella* cytotoxin, *Aeromonas* enterotoxins, toxic shock syndrome toxin-1 (TSST-1), Staphylococcal enterotoxin A (SEA), B (SEB), or C (SEC), Streptococcal enterotoxins and the like. Such bacteria include, but are not limited to, strains of a species of enterotoxigenic *E. coli* (ETEC), enterohemorrhagic *E. coli* (e.g., strains of serotype 0157:H7), *Staphylococcus* species (e.g., *Staphylococcus aureus*, *Staphylococcus pyogenes*), *Shigella* species (e.g., *Shigella dysenteriae*, *Shigella flexneri*, *Shigella boydii*, and *Shigella sonnei*), *Salmonella* species (e.g., *Salmonella typhi*, *Salmonella cholera-suis*, *Salmonella enteritidis*), *Clostridium* species (e.g., *Clostridium perfringens*, *Clostridium difficile*, *Clostridium botulinum*), *Camphlobacter* species (e.g., *Camphlobacter jejuni*, *Camphlobacter fetus*), *Helicobacter* species, (e.g., *Helicobacter pylori*), *Aeromonas* species (e.g., *Aeromonas sobria*, *Aeromonas hydrophila*, *Aeromonas caviae*), *Plesiomonas shigelloides*, *Yersina enterocolitica*, *Vibrios* species (e.g., *Vibrios cholerae*, *Vibrios parahemolyticus*), *Klebsiella* species, *Pseudomonas aeruginosa*, and *Streptococci*. See, e.g., Stein, ed., INTERNAL MEDICINE, 3rd ed., pp 1-13, Little, Brown and Co., Boston, (1990); Evans et al., eds., Bacterial Infections of Humans: Epidemiology and Control, 2d. Ed., pp 239-254, Plenum Medical Book Co., New York (1991); Mandell et al, Principles and Practice of

Infectious Diseases, 3d. Ed., Churchill Livingstone, New York (1990); Berkow et al, eds., *The Merck Manual*, 16th edition, Merck and Co., Rahway, N.J., 1992; Wood et al, FEMS Microbiology Immunology, 76:121-134 (1991); Marrack et al, Science, 248:705-711 (1990), the contents of which references are incorporated entirely herein by reference.

- 5           Anti-TNF antibody compounds, compositions or combinations of the present invention can further comprise at least one of any suitable auxiliary, such as, but not limited to, diluent, binder, stabilizer, buffers, salts, lipophilic solvents, preservative, adjuvant or the like. Pharmaceutically acceptable auxiliaries are preferred. Non-limiting examples of, and methods of preparing such sterile solutions are well known in the art, such as, but limited to, Gennaro, Ed.,  
10    *Remington's Pharmaceutical Sciences*, 18<sup>th</sup> Edition, Mack Publishing Co. (Easton, PA) 1990. Pharmaceutically acceptable carriers can be routinely selected that are suitable for the mode of administration, solubility and/or stability of the anti-TNF antibody, fragment or variant composition as well known in the art or as described herein.

Pharmaceutical excipients and additives useful in the present composition include but are not  
15 limited to proteins, peptides, amino acids, lipids, and carbohydrates (e.g., sugars, including monosaccharides, di-, tri-, tetra-, and oligosaccharides; derivatized sugars such as aldитols, aldonic acids, esterified sugars and the like; and polysaccharides or sugar polymers), which can be present singly or in combination, comprising alone or in combination 1-99.99% by weight or volume. Exemplary protein excipients include serum albumin such as human serum albumin (HSA),  
20 recombinant human albumin (rHA), gelatin, casein, and the like. Representative amino acid/antibody components, which can also function in a buffering capacity, include alanine, glycine, arginine, betaine, histidine, glutamic acid, aspartic acid, cysteine, lysine, leucine, isoleucine, valine, methionine, phenylalanine, aspartame, and the like. One preferred amino acid is glycine.

Carbohydrate excipients suitable for use in the invention include, for example,  
25 monosaccharides such as fructose, maltose, galactose, glucose, D-mannose, sorbose, and the like; disaccharides, such as lactose, sucrose, trehalose, cellobiose, and the like; polysaccharides, such as raffinose, melezitose, maltodextrins, dextrans, starches, and the like; and alditols, such as mannitol, xylitol, maltitol, lactitol, xylitol sorbitol (glucitol), myoinositol and the like. Preferred carbohydrate excipients for use in the present invention are mannitol, trehalose, and raffinose.

30           Anti-TNF antibody compositions can also include a buffer or a pH adjusting agent; typically, the buffer is a salt prepared from an organic acid or base. Representative buffers include organic acid salts such as salts of citric acid, ascorbic acid, gluconic acid, carbonic acid, tartaric acid, succinic

acid, acetic acid, or phthalic acid; Tris, tromethamine hydrochloride, or phosphate buffers. Preferred buffers for use in the present compositions are organic acid salts such as citrate.

Additionally, anti-TNF antibody compositions of the invention can include polymeric excipients/additives such as polyvinylpyrrolidones, ficolls (a polymeric sugar), dextrates (e.g., cyclodextrins, such as 2-hydroxypropyl- $\beta$ -cyclodextrin), polyethylene glycols, flavoring agents, antimicrobial agents, sweeteners, antioxidants, antistatic agents, surfactants (e.g., polysorbates such as "TWEEN 20" and "TWEEN 80"), lipids (e.g., phospholipids, fatty acids), steroids (e.g., cholesterol), and chelating agents (e.g., EDTA).

These and additional known pharmaceutical excipients and/or additives suitable for use in the anti-TNF antibody, portion or variant compositions according to the invention are known in the art, e.g., as listed in "Remington: The Science & Practice of Pharmacy", 19<sup>th</sup> ed., Williams & Williams, (1995), and in the "Physician's Desk Reference", 52<sup>nd</sup> ed., Medical Economics, Montvale, NJ (1998), the disclosures of which are entirely incorporated herein by reference. Preferred carrier or excipient materials are carbohydrates (e.g., saccharides and alditols) and buffers (e.g., citrate) or polymeric agents.

#### Formulations

As noted above, the invention provides for stable formulations, which is preferably a phosphate buffer with saline or a chosen salt, as well as preserved solutions and formulations containing a preservative as well as multi-use preserved formulations suitable for pharmaceutical or veterinary use, comprising at least one anti-TNF antibody in a pharmaceutically acceptable formulation. Preserved formulations contain at least one known preservative or optionally selected from the group consisting of at least one phenol, m-cresol, p-cresol, o-cresol, chlorocresol, benzyl alcohol, phenylmercuric nitrite, phenoxyethanol, formaldehyde, chlorobutanol, magnesium chloride (e.g., hexahydrate), alkylparaben (methyl, ethyl, propyl, butyl and the like), benzalkonium chloride, benzethonium chloride, sodium dehydroacetate and thimerosal, or mixtures thereof in an aqueous diluent. Any suitable concentration or mixture can be used as known in the art, such as 0.001-5%, or any range or value therein, such as, but not limited to 0.001, 0.003, 0.005, 0.009, 0.01, 0.02, 0.03, 0.05, 0.09, 0.1, 0.2, 0.3, 0.4., 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2.0, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, 3.0, 3.1, 3.2, 3.3, 3.4, 3.5, 3.6, 3.7, 3.8, 3.9, 4.0, 4.3, 4.5, 4.6, 4.7, 4.8, 4.9, or any range or value therein. Non-limiting examples include, no preservative, 0.1-2% m-cresol (e.g., 0.2, 0.3, 0.4, 0.5, 0.9, 1.0%), 0.1-3% benzyl alcohol (e.g., 0.5, 0.9, 1.1., 1.5, 1.9, 2.0, 2.5%), 0.001-0.5% thimerosal (e.g., 0.005, 0.01), 0.001-2.0% phenol (e.g., 0.05, 0.25, 0.28, 0.5, 0.9, 1.0%), 0.0005-1.0% alkylparaben(s) (e.g., 0.00075, 0.0009, 0.001, 0.002, 0.005, 0.0075, 0.009, 0.01,

0.02, 0.05, 0.075, 0.09, 0.1, 0.2, 0.3, 0.5, 0.75, 0.9, 1.0%), and the like.

As noted above, the invention provides an article of manufacture, comprising packaging material and at least one vial comprising a solution of at least one anti-TNF antibody with the prescribed buffers and/or preservatives, optionally in an aqueous diluent, wherein said packaging material comprises a label that indicates that such solution can be held over a period of 1, 2, 3, 4, 5, 6, 9, 12, 18, 20, 24, 30, 36, 40, 48, 54, 60, 66, 72 hours or greater. The invention further comprises an article of manufacture, comprising packaging material, a first vial comprising lyophilized at least one anti-TNF antibody, and a second vial comprising an aqueous diluent of prescribed buffer or preservative, wherein said packaging material comprises a label that instructs a patient to reconstitute the at least one anti-TNF antibody in the aqueous diluent to form a solution that can be held over a period of twenty-four hours or greater.

The at least one anti-TNF antibody used in accordance with the present invention can be produced by recombinant means, including from mammalian cell or transgenic preparations, or can be purified from other biological sources, as described herein or as known in the art.

The range of at least one anti-TNF antibody in the product of the present invention includes amounts yielding upon reconstitution, if in a wet/dry system, concentrations from about 1.0 µg/ml to about 1000 mg/ml, although lower and higher concentrations are operable and are dependent on the intended delivery vehicle, e.g., solution formulations will differ from transdermal patch, pulmonary, transmucosal, or osmotic or micro pump methods.

Preferably, the aqueous diluent optionally further comprises a pharmaceutically acceptable preservative. Preferred preservatives include those selected from the group consisting of phenol, m-cresol, p-cresol, o-cresol, chlorocresol, benzyl alcohol, alkylparaben (methyl, ethyl, propyl, butyl and the like), benzalkonium chloride, benzethonium chloride, sodium dehydroacetate and thimerosal, or mixtures thereof. The concentration of preservative used in the formulation is a concentration sufficient to yield an anti-microbial effect. Such concentrations are dependent on the preservative selected and are readily determined by the skilled artisan.

Other excipients, e.g. isotonicity agents, buffers, antioxidants, preservative enhancers, can be optionally and preferably added to the diluent. An isotonicity agent, such as glycerin, is commonly used at known concentrations. A physiologically tolerated buffer is preferably added to provide improved pH control. The formulations can cover a wide range of pHs, such as from about pH 4 to about pH 10, and preferred ranges from about pH 5 to about pH 9, and a most preferred range of about 6.0 to about 8.0. Preferably the formulations of the present invention have pH between about 6.8 and about 7.8. Preferred buffers include phosphate buffers, most preferably sodium phosphate,

particularly phosphate buffered saline (PBS).

- Other additives, such as a pharmaceutically acceptable solubilizers like Tween 20 (polyoxyethylene (20) sorbitan monolaurate), Tween 40 (polyoxyethylene (20) sorbitan monopalmitate), Tween 80 (polyoxyethylene (20) sorbitan monooleate), Pluronic F68 (polyoxyethylene polyoxypropylene block copolymers), and PEG (polyethylene glycol) or non-ionic surfactants such as polysorbate 20 or 80 or poloxamer 184 or 188, Pluronic® polyols, other block copolymers, and chelators such as EDTA and EGTA can optionally be added to the formulations or compositions to reduce aggregation. These additives are particularly useful if a pump or plastic container is used to administer the formulation. The presence of pharmaceutically acceptable surfactant mitigates the propensity for the protein to aggregate.

The formulations of the present invention can be prepared by a process which comprises mixing at least one anti-TNF antibody and a preservative selected from the group consisting of phenol, m-cresol, p-cresol, o-cresol, chlorocresol, benzyl alcohol, alkylparaben, (methyl, ethyl, propyl, butyl and the like), benzalkonium chloride, benzethonium chloride, sodium dehydroacetate and thimerosal or mixtures thereof in an aqueous diluent. Mixing the at least one anti-TNF antibody and preservative in an aqueous diluent is carried out using conventional dissolution and mixing procedures. To prepare a suitable formulation, for example, a measured amount of at least one anti-TNF antibody in buffered solution is combined with the desired preservative in a buffered solution in quantities sufficient to provide the protein and preservative at the desired concentrations. Variations of this process would be recognized by one of ordinary skill in the art. For example, the order the components are added, whether additional additives are used, the temperature and pH at which the formulation is prepared, are all factors that can be optimized for the concentration and means of administration used.

The claimed formulations can be provided to patients as clear solutions or as dual vials comprising a vial of lyophilized at least one anti-TNF antibody that is reconstituted with a second vial containing water, a preservative and/or excipients, preferably a phosphate buffer and/or saline and a chosen salt, in an aqueous diluent. Either a single solution vial or dual vial requiring reconstitution can be reused multiple times and can suffice for a single or multiple cycles of patient treatment and thus can provide a more convenient treatment regimen than currently available.

The present claimed articles of manufacture are useful for administration over a period of immediately to twenty-four hours or greater. Accordingly, the presently claimed articles of manufacture offer significant advantages to the patient. Formulations of the invention can optionally be safely stored at temperatures of from about 2 to about 40°C and retain the biologically activity of

the protein for extended periods of time, thus, allowing a package label indicating that the solution can be held and/or used over a period of 6, 12, 18, 24, 36, 48, 72, or 96 hours or greater. If preserved diluent is used, such label can include use up to 1-12 months, one-half, one and a half, and/or two years.

5        The solutions of at least one anti-TNF antibody in the invention can be prepared by a process that comprises mixing at least one antibody in an aqueous diluent. Mixing is carried out using conventional dissolution and mixing procedures. To prepare a suitable diluent, for example, a measured amount of at least one antibody in water or buffer is combined in quantities sufficient to provide the protein and optionally a preservative or buffer at the desired concentrations. Variations 10 of this process would be recognized by one of ordinary skill in the art. For example, the order the components are added, whether additional additives are used, the temperature and pH at which the formulation is prepared, are all factors that can be optimized for the concentration and means of administration used.

15      The claimed products can be provided to patients as clear solutions or as dual vials comprising a vial of lyophilized at least one anti-TNF antibody that is reconstituted with a second vial containing the aqueous diluent. Either a single solution vial or dual vial requiring reconstitution can be reused multiple times and can suffice for a single or multiple cycles of patient treatment and thus provides a more convenient treatment regimen than currently available.

20      The claimed products can be provided indirectly to patients by providing to pharmacies, clinics, or other such institutions and facilities, clear solutions or dual vials comprising a vial of lyophilized at least one anti-TNF antibody that is reconstituted with a second vial containing the aqueous diluent. The clear solution in this case can be up to one liter or even larger in size, providing a large reservoir from which smaller portions of the at least one antibody solution can be retrieved one or multiple times for transfer into smaller vials and provided by the pharmacy or clinic to their 25 customers and/or patients.

Recognized devices comprising these single vial systems include those pen-injector devices for delivery of a solution such as BD Pens, BD Autojector®, Humaject®, NovoPen®, B-D® Pen, AutoPen®, and OptiPen®, GenotropinPen®, Genotronorm Pen®, Humatro Pen®, Reco-Pen®, Roferon Pen®, Biojector®, Iject®, J-tip Needle-Free Injector®, Inraject®, Medi-Ject®, e.g., as made or 30 developed by Becton Dickensen (Franklin Lakes, NJ, [www.bectondickenson.com](http://www.bectondickenson.com)), Disetronic (Burgdorf, Switzerland, [www.disetronic.com](http://www.disetronic.com); Bioject, Portland, Oregon ([www.bioject.com](http://www.bioject.com)); National Medical Products , Weston Medical (Peterborough, UK, [www.weston-medical.com](http://www.weston-medical.com)), Medi-Ject Corp (Minneapolis, MN, [www.mediject.com](http://www.mediject.com)). Recognized devices comprising a dual vial

system include those pen-injector systems for reconstituting a lyophilized drug in a cartridge for delivery of the reconstituted solution such as the HumatroPen®.

The products presently claimed include packaging material. The packaging material provides, in addition to the information required by the regulatory agencies, the conditions under 5 which the product can be used. The packaging material of the present invention provides instructions to the patient to reconstitute the at least one anti-TNF antibody in the aqueous diluent to form a solution and to use the solution over a period of 2-24 hours or greater for the two vial, wet/dry, product. For the single vial, solution product, the label indicates that such solution can be used over a period of 2-24 hours or greater. The presently claimed products are useful for human pharmaceutical 10 product use.

The formulations of the present invention can be prepared by a process that comprises mixing at least one anti-TNF antibody and a selected buffer, preferably a phosphate buffer containing saline or a chosen salt. Mixing the at least one anti-TNF antibody and buffer in an aqueous diluent is carried out using conventional dissolution and mixing procedures. To prepare a suitable formulation, 15 for example, a measured amount of at least one antibody in water or buffer is combined with the desired buffering agent in water in quantities sufficient to provide the protein and buffer at the desired concentrations. Variations of this process would be recognized by one of ordinary skill in the art. For example, the order the components are added, whether additional additives are used, the temperature and pH at which the formulation is prepared, are all factors that can be optimized for the 20 concentration and means of administration used.

The claimed stable or preserved formulations can be provided to patients as clear solutions or as dual vials comprising a vial of lyophilized at least one anti-TNF antibody that is reconstituted with a second vial containing a preservative or buffer and excipients in an aqueous diluent. Either a single solution vial or dual vial requiring reconstitution can be reused multiple times and can suffice for a 25 single or multiple cycles of patient treatment and thus provides a more convenient treatment regimen than currently available.

Other formulations or methods of stabilizing the anti-TNF antibody may result in other than a clear solution of lyophilized powder comprising said antibody. Among non-clear solutions are formulations comprising particulate suspensions, said particulates being a composition containing the 30 anti-TNF antibody in a structure of variable dimension and known variously as a microsphere, microparticle, nanoparticle, nanosphere, or liposome.

Such relatively homogenous essentially spherical particulate formulations containing an active agent can be formed by contacting an aqueous phase containing the active and a polymer and a

nonaqueous phase followed by evaporation of the nonaqueous phase to cause the coalescence of particles from the aqueous phase as taught in U.S. 4,589,330. Porous microparticles can be prepared using a first phase containing active and a polymer dispersed in a continuous solvent and removing said solvent from the suspension by freeze-drying or dilution-extraction-precipitation as taught in U.S. 4,818,542. Preferred polymers for such preparations are natural or synthetic copolymers or polymer selected from the group consisting of gleatin agar, starch, arabinogalactan, albumin, collagen, polyglycolic acid, polylactic acid, glycolide-L(-) lactide poly(episilon-caprolactone, poly(episilon-caprolactone-CO-lactic acid), poly(episilon-caprolactone-CO-glycolic acid), poly( $\beta$ -hydroxy butyric acid), polyethylene oxide, polyethylene, poly(alkyl-2-cyanoacrylate), poly(hydroxyethyl methacrylate), polyamides, poly(amino acids), poly(2-hydroxyethyl DL-aspartamide), poly(ester urea), poly(L-phenylalanine/ethylene glycol/1,6-diisocyanatohexane) and poly(methyl methacrylate). Particularly preferred polymers are polyesters such as polyglycolic acid, polylactic acid, glycolide-L(-) lactide poly(episilon-caprolactone, poly(episilon-caprolactone-CO-lactic acid), and poly(episilon-caprolactone-CO-glycolic acid). Solvents useful for dissolving the polymer and/or the active include: water, hexafluoroisopropanol, methylenechloride, tetrahydrofuran, hexane, benzene, or hexafluoroacetone sesquihydrate. The process of dispersing the active containing phase with a second phase may include pressure forcing said first phase through an orifice in a nozzle to affect droplet formation.

Dry powder formulations may result from processes other than lyophilization such as by spray drying or solvent extraction by evaporation or by precipitation of a crystalline composition followed by one or more steps to remove aqueous or nonaqueous solvent. Preparation of a spray-dried antibody preparation is taught in U.S. 6,019,968. The antibody-based dry powder compositions may be produced by spray drying solutions or slurries of the antibody and, optionally, excipients, in a solvent under conditions to provide a respirable dry powder. Solvents may include polar compounds such as water and ethanol, which may be readily dried. Antibody stability may be enhanced by performing the spray drying procedures in the absence of oxygen, such as under a nitrogen blanket or by using nitrogen as the drying gas. Another relatively dry formulation is a dispersion of a plurality of perforated microstructures dispersed in a suspension medium that typically comprises a hydrofluoroalkane propellant as taught in WO 9916419. The stabilized dispersions may be administered to the lung of a patient using a metered dose inhaler. Equipment useful in the commercial manufacture of spray dried medicaments are manufactured by Buchi Ltd. or Niro Corp.

At least one anti-TNF antibody in either the stable or preserved formulations or solutions described herein, can be administered to a patient in accordance with the present invention via a

variety of delivery methods including SC or IM injection; transdermal, pulmonary, transmucosal, implant, osmotic pump, cartridge, micro pump, or other means appreciated by the skilled artisan, as well-known in the art.

#### Therapeutic Applications

5 The present invention also provides a method for modulating or treating at least one TNF related disease, in a cell, tissue, organ, animal, or patient, as known in the art or as described herein, using at least one TNF antibody of the present invention.

10 The present invention also provides a method for modulating or treating at least one TNF related disease, in a cell, tissue, organ, animal, or patient including, but not limited to, at least one of obesity, an immune related disease, a cardiovascular disease, an infectious disease, a malignant disease or a neurologic disease.

15 The present invention also provides a method for modulating or treating at least one immune related disease, in a cell, tissue, organ, animal, or patient including, but not limited to, at least one of rheumatoid arthritis, juvenile rheumatoid arthritis, systemic onset juvenile rheumatoid arthritis, psoriatic arthritis, ankylosing spondilitis, gastric ulcer, seronegative arthropathies, osteoarthritis, inflammatory bowel disease, ulcerative colitis, systemic lupus erythematosus, antiphospholipid syndrome, iridocyclitis/uveitis/optic neuritis, idiopathic pulmonary fibrosis, systemic vasculitis/wegener's granulomatosis, sarcoidosis, orchitis/vasectomy reversal procedures, allergic/atopic diseases, asthma, allergic rhinitis, eczema, allergic contact dermatitis, allergic conjunctivitis, hypersensitivity pneumonitis, transplants, organ transplant rejection, graft-versus-host disease, systemic inflammatory response syndrome, sepsis syndrome, gram positive sepsis, gram negative sepsis, culture negative sepsis, fungal sepsis, neutropenic fever, urosepsis, meningococcemia, trauma/hemorrhage, burns, ionizing radiation exposure, acute pancreatitis, adult respiratory distress syndrome, rheumatoid arthritis, alcohol-induced hepatitis, chronic inflammatory pathologies, sarcoidosis, Crohn's pathology, sickle cell anemia, diabetes, nephrosis, atopic diseases, hypersensitivity reactions, allergic rhinitis, hay fever, perennial rhinitis, conjunctivitis, endometriosis, asthma, urticaria, systemic anaphalaxis, dermatitis, pernicious anemia, hemolytic disease, thrombocytopenia, graft rejection of any organ or tissue, kidney transplant rejection, heart transplant rejection, liver transplant rejection, pancreas transplant rejection, lung transplant rejection, bone marrow transplant (BMT) rejection, skin allograft rejection, cartilage transplant rejection, bone graft rejection, small bowel transplant rejection, fetal thymus implant rejection, parathyroid transplant rejection, xenograft rejection of any organ or tissue, allograft rejection, anti-receptor hypersensitivity reactions, Graves disease, Raynoud's disease, type B insulin-resistant diabetes, asthma, myasthenia

gravis, antibody-mediated cytotoxicity, type III hypersensitivity reactions, systemic lupus erythematosus, POEMS syndrome (polyneuropathy, organomegaly, endocrinopathy, monoclonal gammopathy, and skin changes syndrome), polyneuropathy, organomegaly, endocrinopathy, monoclonal gammopathy, skin changes syndrome, antiphospholipid syndrome, pemphigus,  
5 scleroderma, mixed connective tissue disease, idiopathic Addison's disease, diabetes mellitus, chronic active hepatitis, primary biliary cirrhosis, vitiligo, vasculitis, post-MI cardiotomy syndrome, type IV hypersensitivity, contact dermatitis, hypersensitivity pneumonitis, allograft rejection, granulomas due to intracellular organisms, drug sensitivity, metabolic/idiopathic, Wilson's disease, hemachromatosis, alpha-1-antitrypsin deficiency, diabetic retinopathy, hashimoto's thyroiditis,  
10 osteoporosis, hypothalamic-pituitary-adrenal axis evaluation, primary biliary cirrhosis, thyroiditis, encephalomyelitis, cachexia, cystic fibrosis, neonatal chronic lung disease, chronic obstructive pulmonary disease (COPD), familial hematophagocytic lymphohistiocytosis, dermatologic conditions, psoriasis, alopecia, nephrotic syndrome, nephritis, glomerular nephritis, acute renal failure, hemodialysis, uremia, toxicity, preeclampsia, okt3 therapy, anti-cd3 therapy, cytokine  
15 therapy, chemotherapy, radiation therapy (e.g., including but not limited to asthenia, anemia, cachexia, and the like), chronic salicylate intoxication, and the like. See, e.g., the Merck Manual, 12th-17th Editions, Merck & Company, Rahway, NJ (1972, 1977, 1982, 1987, 1992, 1999), Pharmacotherapy Handbook, Wells et al., eds., Second Edition, Appleton and Lange, Stamford, Conn. (1998, 2000), each entirely incorporated by reference.

20 The present invention also provides a method for modulating or treating at least one cardiovascular disease in a cell, tissue, organ, animal, or patient, including, but not limited to, at least one of cardiac stun syndrome, myocardial infarction, congestive heart failure, stroke, ischemic stroke, hemorrhage, arteriosclerosis, atherosclerosis, restenosis, diabetic atherosclerotic disease, hypertension, arterial hypertension, renovascular hypertension, syncope, shock, syphilis of the  
25 cardiovascular system, heart failure, cor pulmonale, primary pulmonary hypertension, cardiac arrhythmias, atrial ectopic beats, atrial flutter, atrial fibrillation (sustained or paroxysmal), post perfusion syndrome, cardiopulmonary bypass inflammation response, chaotic or multifocal atrial tachycardia, regular narrow QRS tachycardia, specific arrhythmias, ventricular fibrillation, His bundle arrhythmias, atrioventricular block, bundle branch block, myocardial ischemic disorders, coronary  
30 artery disease, angina pectoris, myocardial infarction, cardiomyopathy, dilated congestive cardiomyopathy, restrictive cardiomyopathy, valvular heart diseases, endocarditis, pericardial disease, cardiac tumors, aortic and peripheral aneurysms, aortic dissection, inflammation of the aorta, occlusion of the abdominal aorta and its branches, peripheral vascular disorders, occlusive

arterial disorders, peripheral atherosclerotic disease, thromboangiitis obliterans, functional peripheral arterial disorders, Raynaud's phenomenon and disease, acrocyanosis, erythromelalgia, venous diseases, venous thrombosis, varicose veins, arteriovenous fistula, lymphedema, lipedema, unstable angina, reperfusion injury, post pump syndrome, ischemia-reperfusion injury, and the like. Such a 5 method can optionally comprise administering an effective amount of a composition or pharmaceutical composition comprising at least one anti-TNF antibody to a cell, tissue, organ, animal or patient in need of such modulation, treatment or therapy.

The present invention also provides a method for modulating or treating at least one infectious disease in a cell, tissue, organ, animal or patient, including, but not limited to, at least one 10 of: acute or chronic bacterial infection, acute and chronic parasitic or infectious processes, including bacterial, viral and fungal infections, HIV infection/HIV neuropathy, meningitis, hepatitis (e.g., A,B or C, or the like), septic arthritis, peritonitis, pneumonia, epiglottitis, e. coli O157:h7, hemolytic uremic syndrome/thrombolytic thrombocytopenic purpura, malaria, dengue hemorrhagic fever, leishmaniasis, leprosy, toxic shock syndrome, streptococcal myositis, gas gangrene, mycobacterium 15 tuberculosis, mycobacterium avium intracellulare, pneumocystis carinii pneumonia, pelvic inflammatory disease, orchitis/epididymitis, legionella, lyme disease, influenza a, epstein-barr virus, vital-associated hemaphagocytic syndrome, vital encephalitis/aseptic meningitis, and the like;

The present invention also provides a method for modulating or treating at least one malignant disease in a cell, tissue, organ, animal or patient, including, but not limited to, at least one 20 of: leukemia, acute leukemia, acute lymphoblastic leukemia (ALL), B-cell, T-cell or FAB ALL, acute myeloid leukemia (AML), chronic myelocytic leukemia (CML), chronic lymphocytic leukemia (CLL), hairy cell leukemia, myelodysplastic syndrome (MDS), a lymphoma, Hodgkin's disease, a malignant lymphoma, non-hodgkin's lymphoma, Burkitt's lymphoma, multiple myeloma, Kaposi's sarcoma, colorectal carcinoma, pancreatic carcinoma, nasopharyngeal carcinoma, malignant 25 histiocytosis, paraneoplastic syndrome/hypercalcemia of malignancy, solid tumors, adenocarcinomas, sarcomas, malignant melanoma, hemangioma, metastatic disease, cancer related bone resorption, cancer related bone pain, and the like.

The present invention also provides a method for modulating or treating at least one neurologic disease in a cell, tissue, organ, animal or patient, including, but not limited to, at least one 30 of: neurodegenerative diseases, multiple sclerosis, migraine headache, AIDS dementia complex, demyelinating diseases, such as multiple sclerosis and acute transverse myelitis; extrapyramidal and cerebellar disorders' such as lesions of the corticospinal system; disorders of the basal ganglia or

cerebellar disorders; hyperkinetic movement disorders such as Huntington's Chorea and senile chorea; drug-induced movement disorders, such as those induced by drugs which block CNS dopamine receptors; hypokinetic movement disorders, such as Parkinson's disease; Progressive supra-nucleo Palsy; structural lesions of the cerebellum; spinocerebellar degenerations, such as spinal ataxia, Friedreich's ataxia, cerebellar cortical degenerations, multiple systems degenerations (Mencel, Dejerine-Thomas, Shi-Drager, and Machado-Joseph); systemic disorders (Refsum's disease, abetalipoproteinemia, ataxia, telangiectasia, and mitochondrial multi-system disorder); demyelinating core disorders, such as multiple sclerosis, acute transverse myelitis; and disorders of the motor unit such as neurogenic muscular atrophies (anterior horn cell degeneration, such as amyotrophic lateral sclerosis, infantile spinal muscular atrophy and juvenile spinal muscular atrophy); Alzheimer's disease; Down's Syndrome in middle age; Diffuse Lewy body disease; Senile Dementia of Lewy body type; Wernicke-Korsakoff syndrome; chronic alcoholism; Creutzfeldt-Jakob disease; Subacute sclerosing panencephalitis, Hallervorden-Spatz disease; and Dementia pugilistica, and the like. Such a method can optionally comprise administering an effective amount of a composition or

15 pharmaceutical composition comprising at least one TNF antibody or specified portion or variant to a cell, tissue, organ, animal or patient in need of such modulation, treatment or therapy. See, e.g., the Merck Manual, 16<sup>th</sup> Edition, Merck & Company, Rahway, NJ (1992)

Any method of the present invention can comprise administering an effective amount of a composition or pharmaceutical composition comprising at least one anti-TNF antibody to a cell, tissue, organ, animal or patient in need of such modulation, treatment or therapy. Such a method can optionally further comprise co-administration or combination therapy for treating such immune diseases, wherein the administering of said at least one anti-TNF antibody, specified portion or variant thereof, further comprises administering, before concurrently, and/or after, at least one selected from at least one TNF antagonist (e.g., but not limited to a TNF antibody or fragment, a soluble TNF receptor or fragment, fusion proteins thereof, or a small molecule TNF antagonist), an antirheumatic (e.g., methotrexate, auranofin, aurothioglucose, azathioprine, etanercept, gold sodium thiomalate, hydroxychloroquine sulfate, leflunomide, sulfasalazine), a muscle relaxant, a narcotic, a non-steroid anti-inflammatory drug (NSAID), an analgesic, an anesthetic, a sedative, a local anesthetic, a neuromuscular blocker, an antimicrobial (e.g., aminoglycoside, an antifungal, an antiparasitic, an antiviral, a carbapenem, cephalosporin, a flurorquinolone, a macrolide, a penicillin, a sulfonamide, a tetracycline, another antimicrobial), an antipsoriatic, a corticosteroid, an anabolic steroid, a diabetes related agent, a mineral, a nutritional, a thyroid agent, a vitamin, a calcium related hormone, an antidiarrheal, an antitussive, an antiemetic, an antiulcer, a laxative, an anticoagulant, an

erythropoietin (e.g., epoetin alpha), a filgrastim (e.g., G-CSF, Neupogen), a sargramostim (GM-CSF, Leukine), an immunization, an immunoglobulin, an immunosuppressive (e.g., basiliximab, cyclosporine, daclizumab), a growth hormone, a hormone replacement drug, an estrogen receptor modulator, a mydriatic, a cycloplegic, an alkylating agent, an antimetabolite, a mitotic inhibitor, a radiopharmaceutical, an antidepressant, antimanic agent, an antipsychotic, an anxiolytic, a hypnotic, a sympathomimetic, a stimulant, donepezil, tacrine, an asthma medication, a beta agonist, an inhaled steroid, a leukotriene inhibitor, a methylxanthine, a cromolyn, an epinephrine or analog, dornase alpha (Pulmozyme), a cytokine or a cytokine antagonist. Suitable dosages are well known in the art. See, e.g., Wells et al., eds., *Pharmacotherapy Handbook*, 2<sup>nd</sup> Edition, Appleton and Lange, Stamford, CT (2000); PDR *Pharmacopoeia*, Tarascon *Pocket Pharmacopoeia 2000*, Deluxe Edition, Tarascon Publishing, Loma Linda, CA (2000), each of which references are entirely incorporated herein by reference.

TNF antagonists suitable for compositions, combination therapy, co-administration, devices and/or methods of the present invention (further comprising at least one anti body, specified portion and variant thereof, of the present invention), include, but are not limited to, anti-TNF antibodies, antigen-binding fragments thereof, and receptor molecules which bind specifically to TNF; compounds which prevent and/or inhibit TNF synthesis, TNF release or its action on target cells, such as thalidomide, tenidap, phosphodiesterase inhibitors (e.g., pentoxifylline and rolipram), A2b adenosine receptor agonists and A2b adenosine receptor enhancers; compounds which prevent and/or inhibit TNF receptor signalling, such as mitogen activated protein (MAP) kinase inhibitors; compounds which block and/or inhibit membrane TNF cleavage, such as metalloproteinase inhibitors; compounds which block and/or inhibit TNF activity, such as angiotensin converting enzyme (ACE) inhibitors (e.g., captopril); and compounds which block and/or inhibit TNF production and/or synthesis, such as MAP kinase inhibitors.

As used herein, a "tumor necrosis factor antibody," "TNF antibody," "TNF $\alpha$  antibody," or fragment and the like decreases, blocks, inhibits, abrogates or interferes with TNF $\alpha$  activity *in vitro*, *in situ* and/or preferably *in vivo*. For example, a suitable TNF human antibody of the present invention can bind TNF $\alpha$  and includes anti-TNF antibodies, antigen-binding fragments thereof, and specified mutants or domains thereof that bind specifically to TNF $\alpha$ . A suitable TNF antibody or fragment can also decrease block, abrogate, interfere, prevent and/or inhibit TNF RNA, DNA or protein synthesis, TNF release, TNF receptor signaling, membrane TNF cleavage, TNF activity, TNF production and/or synthesis.

Chimeric antibody cA2 consists of the antigen binding variable region of the high-affinity

neutralizing mouse anti-human TNF $\alpha$  IgG1 antibody, designated A2, and the constant regions of a human IgG1, kappa immunoglobulin. The human IgG1 Fc region improves allogeneic antibody effector function, increases the circulating serum half-life and decreases the immunogenicity of the antibody. The avidity and epitope specificity of the chimeric antibody cA2 is derived from the 5 variable region of the murine antibody A2. In a particular embodiment, a preferred source for nucleic acids encoding the variable region of the murine antibody A2 is the A2 hybridoma cell line.

Chimeric A2 (cA2) neutralizes the cytotoxic effect of both natural and recombinant human TNF $\alpha$  in a dose dependent manner. From binding assays of chimeric antibody cA2 and recombinant human TNF $\alpha$ , the affinity constant of chimeric antibody cA2 was calculated to be  $1.04 \times 10^{10} M^{-1}$ .  
10 Preferred methods for determining monoclonal antibody specificity and affinity by competitive inhibition can be found in Harlow, *et al.*, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 1988; Colligan *et al.*, eds., *Current Protocols in Immunology*, Greene Publishing Assoc. and Wiley Interscience, New York, (1992-2000); Kozbor *et al.*, *Immunol. Today*, 4:72-79 (1983); Ausubel *et al.*, eds. *Current Protocols in Molecular Biology*,  
15 Wiley Interscience, New York (1987-2000); and Muller, *Meth. Enzymol.*, 92:589-601 (1983), which references are entirely incorporated herein by reference.

In a particular embodiment, murine monoclonal antibody A2 is produced by a cell line designated c134A. Chimeric antibody cA2 is produced by a cell line designated c168A.

Additional examples of monoclonal anti-TNF antibodies that can be used in the present  
20 invention are described in the art (see, e.g., U.S. Patent No. 5,231,024; Möller, A. *et al.*, *Cytokine* 2(3):162-169 (1990); U.S. Application No. 07/943,852 (filed September 11, 1992); Rathjen *et al.*, International Publication No. WO 91/02078 (published February 21, 1991); Rubin *et al.*, EPO Patent Publication No. 0 218 868 (published April 22, 1987); Yone *et al.*, EPO Patent Publication No. 0 288 088 (October 26, 1988); Liang, *et al.*, *Biochem. Biophys. Res. Comm.* 137:847-854 (1986); Meager, *et al.*, *Hybridoma* 6:305-311 (1987); Fendly *et al.*, *Hybridoma* 6:359-369 (1987); Bringman, *et al.*, *Hybridoma* 6:489-507 (1987); and Hirai, *et al.*, *J. Immunol. Meth.* 96:57-62 (1987), which references are entirely incorporated herein by reference).

#### TNF Receptor Molecules

30 Preferred TNF receptor molecules useful in the present invention are those that bind TNF $\alpha$  with high affinity (see, e.g., Feldmann *et al.*, International Publication No. WO 92/07076 (published April 30, 1992); Schall *et al.*, *Cell* 61:361-370 (1990); and Loetscher *et al.*, *Cell* 61:351-359 (1990), which references are entirely incorporated herein by reference) and optionally possess low

immunogenicity. In particular, the 55 kDa (p55 TNF-R) and the 75 kDa (p75 TNF-R) TNF cell surface receptors are useful in the present invention. Truncated forms of these receptors, comprising the extracellular domains (ECD) of the receptors or functional portions thereof (see, e.g., Corcoran *et al.*, *Eur. J. Biochem.* 223:831-840 (1994)), are also useful in the present invention. Truncated forms 5 of the TNF receptors, comprising the ECD, have been detected in urine and serum as 30 kDa and 40 kDa TNF $\alpha$  inhibitory binding proteins (Engelmann, H. *et al.*, *J. Biol. Chem.* 265:1531-1536 (1990)). TNF receptor multimeric molecules and TNF immunoreceptor fusion molecules, and derivatives and fragments or portions thereof, are additional examples of TNF receptor molecules which are useful in the methods and compositions of the present invention. The TNF receptor molecules which can be 10 used in the invention are characterized by their ability to treat patients for extended periods with good to excellent alleviation of symptoms and low toxicity. Low immunogenicity and/or high affinity, as well as other undefined properties, can contribute to the therapeutic results achieved.

TNF receptor multimeric molecules useful in the present invention comprise all or a functional portion of the ECD of two or more TNF receptors linked via one or more polypeptide 15 linkers or other nonpeptide linkers, such as polyethylene glycol (PEG). The multimeric molecules can further comprise a signal peptide of a secreted protein to direct expression of the multimeric molecule. These multimeric molecules and methods for their production have been described in U.S. Application No. 08/437,533 (filed May 9, 1995), the content of which is entirely incorporated herein by reference.

20 TNF immunoreceptor fusion molecules useful in the methods and compositions of the present invention comprise at least one portion of one or more immunoglobulin molecules and all or a functional portion of one or more TNF receptors. These immunoreceptor fusion molecules can be assembled as monomers, or hetero- or homo-multimers. The immunoreceptor fusion molecules can also be monovalent or multivalent. An example of such a TNF immunoreceptor fusion molecule is 25 TNF receptor/IgG fusion protein. TNF immunoreceptor fusion molecules and methods for their production have been described in the art (Lesslauer *et al.*, *Eur. J. Immunol.* 21:2883-2886 (1991); Ashkenazi *et al.*, *Proc. Natl. Acad. Sci. USA* 88:10535-10539 (1991); Peppel *et al.*, *J. Exp. Med.* 174:1483-1489 (1991); Kolls *et al.*, *Proc. Natl. Acad. Sci. USA* 91:215-219 (1994); Butler *et al.*, *Cytokine* 6(6):616-623 (1994); Baker *et al.*, *Eur. J. Immunol.* 24:2040-2048 (1994); Beutler *et al.*, 30 U.S. Patent No. 5,447,851; and U.S. Application No. 08/442,133 (filed May 16, 1995), each of which references are entirely incorporated herein by reference). Methods for producing immunoreceptor fusion molecules can also be found in Capon *et al.*, U.S. Patent No. 5,116,964; Capon *et al.*, U.S. Patent No. 5,225,538; and Capon *et al.*, *Nature* 337:525-531 (1989), which references are entirely

incorporated herein by reference.

A functional equivalent, derivative, fragment or region of TNF receptor molecule refers to the portion of the TNF receptor molecule, or the portion of the TNF receptor molecule sequence which encodes TNF receptor molecule, that is of sufficient size and sequences to functionally

- 5 resemble TNF receptor molecules that can be used in the present invention (e.g., bind TNF $\alpha$  with high affinity and possess low immunogenicity). A functional equivalent of TNF receptor molecule also includes modified TNF receptor molecules that functionally resemble TNF receptor molecules that can be used in the present invention (e.g., bind TNF $\alpha$  with high affinity and possess low immunogenicity). For example, a functional equivalent of TNF receptor molecule can contain a  
10 "SILENT" codon or one or more amino acid substitutions, deletions or additions (e.g., substitution of one acidic amino acid for another acidic amino acid; or substitution of one codon encoding the same or different hydrophobic amino acid for another codon encoding a hydrophobic amino acid). See Ausubel *et al.*, *Current Protocols in Molecular Biology*, Greene Publishing Assoc. and Wiley-Interscience, New York (1987-2000).

- 15 Cytokines include any known cytokine. See, e.g., [www.Copewithcytokines.de](http://www.Copewithcytokines.de). Cytokine antagonists include, but are not limited to, any antibody, fragment or mimetic, any soluble receptor, fragment or mimetic, any small molecule antagonist, or any combination thereof.

**Therapeutic Treatments.**

- Any method of the present invention can comprise a method for treating a TNF mediated disorder, comprising administering an effective amount of a composition or pharmaceutical composition comprising at least one anti-TNF antibody to a cell, tissue, organ, animal or patient in need of such modulation, treatment or therapy. Such a method can optionally further comprise co-administration or combination therapy for treating such immune diseases, wherein the administering of said at least one anti-TNF antibody, specified portion or variant thereof, further comprises  
20 administering, before concurrently, and/or after, at least one selected from at least one at least one selected from at least one TNF antagonist (e.g., but not limited to a TNF antibody or fragment, a soluble TNF receptor or fragment, fusion proteins thereof, or a small molecule TNF antagonist), an antirheumatic (e.g., methotrexate, auranofin, aurothioglucose, azathioprine, etanercept, gold sodium thiomalate, hydroxychloroquine sulfate, leflunomide, sulfasalazine), a muscle relaxant, a narcotic, a  
25 non-steroid anti-inflammatory drug (NSAID), an analgesic, an anesthetic, a sedative, a local anesthetic, a neuromuscular blocker, an antimicrobial (e.g., aminoglycoside, an antifungal, an antiparasitic, an antiviral, a carbapenem, cephalosporin, a flurorquinolone, a macrolide, a penicillin, a sulfonamide, a tetracycline, another antimicrobial), an antipsoriatic, a corticosteroid, an anabolic  
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steroid, a diabetes related agent, a mineral, a nutritional, a thyroid agent, a vitamin, a calcium related hormone, an antidiarrheal, an antitussive, an antiemetic, an antiulcer, a laxative, an anticoagulant, an erythropoietin (e.g., epoetin alpha), a filgrastim (e.g., G-CSF, Neupogen), a sargramostim (GM-CSF, Leukine), an immunization, an immunoglobulin, an immunosuppressive (e.g., basiliximab,  
5 cyclosporine, daclizumab), a growth hormone, a hormone replacement drug, an estrogen receptor modulator, a mydriatic, a cycloplegic, an alkylating agent, an antimetabolite, a mitotic inhibitor, a radiopharmaceutical, an antidepressant, antimanic agent, an antipsychotic, an anxiolytic, a hypnotic, a sympathomimetic, a stimulant, donepezil, tacrine, an asthma medication, a beta agonist, an inhaled steroid, a leukotriene inhibitor, a methylxanthine, a cromolyn, an epinephrine or analog, dornase  
10 alpha (Pulmozyme), a cytokine or a cytokine antagonist.

Typically, treatment of pathologic conditions is effected by administering an effective amount or dosage of at least one anti-TNF antibody composition that total, on average, a range from at least about 0.01 to 500 milligrams of at least one anti-TNF antibody per kilogram of patient per dose, and preferably from at least about 0.1 to 100 milligrams antibody /kilogram of patient per single or multiple  
15 administration, depending upon the specific activity of contained in the composition. Alternatively, the effective serum concentration can comprise 0.1-5000 µg/ml serum concentration per single or multiple administration. Suitable dosages are known to medical practitioners and will, of course, depend upon the particular disease state, specific activity of the composition being administered, and the particular patient undergoing treatment. In some instances, to achieve the desired therapeutic amount, it can be  
20 necessary to provide for repeated administration, *i.e.*, repeated individual administrations of a particular monitored or metered dose, where the individual administrations are repeated until the desired daily dose or effect is achieved.

Preferred doses can optionally include 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35,  
25 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 and/or 100-500 mg/kg/administration, or any range, value or fraction thereof, or to achieve a serum concentration of 0.1, 0.5, 0.9, 1.0, 1.1, 1.2, 1.5, 1.9, 2.0, 2.5, 2.9, 3.0, 3.5, 3.9, 4.0, 4.5, 4.9, 5.0, 5.5, 5.9, 6.0, 6.5, 6.9, 7.0, 7.5, 7.9, 8.0, 8.5, 8.9, 9.0, 9.5, 9.9, 10, 10.5, 10.9, 11, 11.5, 11.9, 30 20, 12.5, 12.9, 13.0, 13.5, 13.9, 14.0, 14.5, 4.9, 5.0, 5.5., 5.9, 6.0, 6.5, 6.9, 7.0, 7.5, 7.9, 8.0, 8.5, 8.9, 9.0, 9.5, 9.9, 10, 10.5, 10.9, 11, 11.5, 11.9, 12, 12.5, 12.9, 13.0, 13.5, 13.9, 14, 14.5, 15, 15.5, 15.9, 16, 16.5, 16.9, 17, 17.5, 17.9, 18, 18.5, 18.9, 19, 19.5, 19.9, 20, 20.5, 20.9, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 96, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000,

1500, 2000, 2500, 3000, 3500, 4000, 4500, and/or 5000 µg/ml serum concentration per single or multiple administration, or any range, value or fraction thereof.

Alternatively, the dosage administered can vary depending upon known factors, such as the pharmacodynamic characteristics of the particular agent, and its mode and route of administration;

- 5 age, health, and weight of the recipient; nature and extent of symptoms, kind of concurrent treatment, frequency of treatment, and the effect desired. Usually a dosage of active ingredient can be about 0.1 to 100 milligrams per kilogram of body weight. Ordinarily 0.1 to 50, and preferably 0.1 to 10 milligrams per kilogram per administration or in sustained release form is effective to obtain desired results.

- 10 As a non-limiting example, treatment of humans or animals can be provided as a one-time or periodic dosage of at least one antibody of the present invention 0.1 to 100 mg/kg, such as 0.5, 0.9, 1.0, 1.1, 1.5, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 40, 45, 50, 60, 70, 80, 90 or 100 mg/kg, per day, on at least one of day 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, or 40, or alternatively or additionally, at least one of week 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, or 52, or alternatively or additionally, at least one of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 years, or any combination thereof, using single, infusion or repeated doses.

- 20 Dosage forms (composition) suitable for internal administration generally contain from about 0.1 milligram to about 500 milligrams of active ingredient per unit or container. In these pharmaceutical compositions the active ingredient will ordinarily be present in an amount of about 0.5-99.999% by weight based on the total weight of the composition.

- 25 For parenteral administration, the antibody can be formulated as a solution, suspension, emulsion, particle, powder, or lyophilized powder in association, or separately provided, with a pharmaceutically acceptable parenteral vehicle. Examples of such vehicles are water, saline, Ringer's solution, dextrose solution, and 1-10% human serum albumin. Liposomes and nonaqueous vehicles such as fixed oils can also be used. The vehicle or lyophilized powder can contain additives that maintain isotonicity (e.g., sodium chloride, mannitol) and chemical stability (e.g., buffers and preservatives). The formulation is sterilized by known or suitable techniques.

Suitable pharmaceutical carriers are described in the most recent edition of Remington's Pharmaceutical Sciences, A. Osol, a standard reference text in this field.

#### **Alternative Administration**

Many known and developed modes of can be used according to the present invention for administering pharmaceutically effective amounts of at least one anti-TNF antibody according to the present invention. While pulmonary administration is used in the following description, other modes of administration can be used according to the present invention with suitable results.

- 5 TNF antibodies of the present invention can be delivered in a carrier, as a solution, emulsion, colloid, or suspension, or as a dry powder, using any of a variety of devices and methods suitable for administration by inhalation or other modes described here within or known in the art.

#### **Parenteral Formulations and Administration**

Formulations for parenteral administration can contain as common excipients sterile water or saline, polyalkylene glycols such as polyethylene glycol, oils of vegetable origin, hydrogenated naphthalenes and the like. Aqueous or oily suspensions for injection can be prepared by using an appropriate emulsifier or humidifier and a suspending agent, according to known methods. Agents for injection can be a non-toxic, non-orally administrable diluting agent such as aqueous solution or a sterile injectable solution or suspension in a solvent. As the usable vehicle or solvent, water, Ringer's solution, isotonic saline, etc. are allowed; as an ordinary solvent, or suspending solvent, sterile involatile oil can be used. For these purposes, any kind of involatile oil and fatty acid can be used, including natural or synthetic or semisynthetic fatty oils or fatty acids; natural or synthetic or semisynthetic mono- or di- or tri-glycerides. Parental administration is known in the art and includes, but is not limited to, conventional means of injections, a gas pressured needle-less injection device as described in U.S. Pat. No. 5,851,198, and a laser perforator device as described in U.S. Pat. No. 5,839,446 entirely incorporated herein by reference.

#### **Alternative Delivery**

The invention further relates to the administration of at least one anti-TNF antibody by parenteral, subcutaneous, intramuscular, intravenous, intrarticular, intrabronchial, intraabdominal, 25 intracapsular, intracartilaginous, intracavitory, intracelial, intracelebellar, intracerebroventricular, intracolic, intracervical, intragastric, intrahepatic, intramyocardial, intraosteal, intrapelvic, intrapericardiac, intraperitoneal, intrapleural, intraprostatic, intrapulmonary, intrarectal, intrarenal, intraretinal, intraspinal, intrasynovial, intrathoracic, intrauterine, intravesical, bolus, vaginal, rectal, buccal, sublingual, intranasal, or transdermal means. At least one anti-TNF antibody composition can 30 be prepared for use for parenteral (subcutaneous, intramuscular or intravenous) or any other administration particularly in the form of liquid solutions or suspensions; for use in vaginal or rectal administration particularly in semisolid forms such as, but not limited to, creams and suppositories; for buccal, or sublingual administration such as, but not limited to, in the form of tablets or capsules;

- or intranasally such as, but not limited to, the form of powders, nasal drops or aerosols or certain agents; or transdermally such as not limited to a gel, ointment, lotion, suspension or patch delivery system with chemical enhancers such as dimethyl sulfoxide to either modify the skin structure or to increase the drug concentration in the transdermal patch (Junginger, et al. In "Drug Permeation Enhancement"; Hsieh, D. S., Eds., pp. 59-90 (Marcel Dekker, Inc. New York 1994, entirely incorporated herein by reference), or with oxidizing agents that enable the application of formulations containing proteins and peptides onto the skin (WO 98/53847), or applications of electric fields to create transient transport pathways such as electroporation, or to increase the mobility of charged drugs through the skin such as iontophoresis, or application of ultrasound such as sonophoresis (U.S. Pat. Nos. 4,309,989 and 4,767,402) (the above publications and patents being entirely incorporated herein by reference).

#### Pulmonary/Nasal Administration

For pulmonary administration, preferably at least one anti-TNF antibody composition is delivered in a particle size effective for reaching the lower airways of the lung or sinuses. According to the invention, at least one anti-TNF antibody can be delivered by any of a variety of inhalation or nasal devices known in the art for administration of a therapeutic agent by inhalation. These devices capable of depositing aerosolized formulations in the sinus cavity or alveoli of a patient include metered dose inhalers, nebulizers, dry powder generators, sprayers, and the like. Other devices suitable for directing the pulmonary or nasal administration of antibodies are also known in the art.

All such devices can use of formulations suitable for the administration for the dispensing of antibody in an aerosol. Such aerosols can be comprised of either solutions (both aqueous and non aqueous) or solid particles. Metered dose inhalers like the Ventolin® metered dose inhaler, typically use a propellant gas and require actuation during inspiration (See, e.g., WO 94/16970, WO 98/35888). Dry powder inhalers like Turbuhaler™ (Astra), Rotahaler® (Glaxo), Diskus® (Glaxo), Spiros™ inhaler (Dura), devices marketed by Inhale Therapeutics, and the Spinhaler® powder inhaler (Fisons), use breath-actuation of a mixed powder (US 4668218 Astra, EP 237507 Astra, WO 97/25086 Glaxo, WO 94/08552 Dura, US 5458135 Inhale, WO 94/06498 Fisons, entirely incorporated herein by reference). Nebulizers like AERx™ Aradigm, the Ultravent® nebulizer (Mallinckrodt), and the Acorn II® nebulizer (Marquest Medical Products) (US 5404871 Aradigm, WO 97/22376), the above references entirely incorporated herein by reference, produce aerosols from solutions, while metered dose inhalers, dry powder inhalers, etc. generate small particle aerosols. These specific examples of commercially available inhalation devices are intended to be a representative of specific devices suitable for the practice of this invention, and are not intended as

limiting the scope of the invention. Preferably, a composition comprising at least one anti-TNF antibody is delivered by a dry powder inhaler or a sprayer. There are several desirable features of an inhalation device for administering at least one antibody of the present invention. For example, delivery by the inhalation device is advantageously reliable, reproducible, and accurate. The 5 inhalation device can optionally deliver small dry particles, e.g. less than about 10  $\mu\text{m}$ , preferably about 1-5  $\mu\text{m}$ , for good respirability.

#### Administration of TNF antibody Compositions as a Spray

A spray including TNF antibody composition can be produced by forcing a suspension or solution of at least one anti-TNF antibody through a nozzle under pressure. The nozzle size and 10 configuration, the applied pressure, and the liquid feed rate can be chosen to achieve the desired output and particle size. An electrospray can be produced, for example, by an electric field in connection with a capillary or nozzle feed. Advantageously, particles of at least one anti-TNF antibody composition delivered by a sprayer have a particle size less than about 10  $\mu\text{m}$ , preferably in the range of about 1  $\mu\text{m}$  to about 5  $\mu\text{m}$ , and most preferably about 2  $\mu\text{m}$  to about 3  $\mu\text{m}$ .

15 Formulations of at least one anti-TNF antibody composition suitable for use with a sprayer typically include antibody composition in an aqueous solution at a concentration of about 0.1 mg to about 100 mg of at least one anti-TNF antibody composition per ml of solution or mg/gm, or any range or value therein, e.g., but not limited to, .1, .2, .3, .4, .5, .6, .7, .8, .9, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 40, 45, 50, 60, 70, 80, 90 20 or 100 mg/ml or mg/gm. The formulation can include agents such as an excipient, a buffer, an isotonicity agent, a preservative, a surfactant, and, preferably, zinc. The formulation can also include an excipient or agent for stabilization of the antibody composition, such as a buffer, a reducing agent, a bulk protein, or a carbohydrate. Bulk proteins useful in formulating antibody compositions include albumin, protamine, or the like. Typical carbohydrates useful in formulating antibody compositions 25 include sucrose, mannitol, lactose, trehalose, glucose, or the like. The antibody composition formulation can also include a surfactant, which can reduce or prevent surface-induced aggregation of the antibody composition caused by atomization of the solution in forming an aerosol. Various conventional surfactants can be employed, such as polyoxyethylene fatty acid esters and alcohols, and polyoxyethylene sorbitol fatty acid esters. Amounts will generally range between 0.001 and 14% 30 by weight of the formulation. Especially preferred surfactants for purposes of this invention are polyoxyethylene sorbitan monooleate, polysorbate 80, polysorbate 20, or the like. Additional agents known in the art for formulation of a protein such as TNF antibodies, or specified portions or variants, can also be included in the formulation.

**Administration of TNF antibody compositions by a Nebulizer**

antibody composition can be administered by a nebulizer, such as jet nebulizer or an ultrasonic nebulizer. Typically, in a jet nebulizer, a compressed air source is used to create a high-velocity air jet through an orifice. As the gas expands beyond the nozzle, a low-pressure region is created, which draws a solution of antibody composition through a capillary tube connected to a liquid reservoir. The liquid stream from the capillary tube is sheared into unstable filaments and droplets as it exits the tube, creating the aerosol. A range of configurations, flow rates, and baffle types can be employed to achieve the desired performance characteristics from a given jet nebulizer. In an ultrasonic nebulizer, high-frequency electrical energy is used to create vibrational, mechanical energy, typically employing a piezoelectric transducer. This energy is transmitted to the formulation of antibody composition either directly or through a coupling fluid, creating an aerosol including the antibody composition. Advantageously, particles of antibody composition delivered by a nebulizer have a particle size less than about 10  $\mu\text{m}$ , preferably in the range of about 1  $\mu\text{m}$  to about 5  $\mu\text{m}$ , and most preferably about 2  $\mu\text{m}$  to about 3  $\mu\text{m}$ .

Formulations of at least one anti-TNF antibody suitable for use with a nebulizer, either jet or ultrasonic, typically include a concentration of about 0.1 mg to about 100 mg of at least one anti-TNF antibody protein per ml of solution. The formulation can include agents such as an excipient, a buffer, an isotonicity agent, a preservative, a surfactant, and, preferably, zinc. The formulation can also include an excipient or agent for stabilization of the at least one anti-TNF antibody composition, such as a buffer, a reducing agent, a bulk protein, or a carbohydrate. Bulk proteins useful in formulating at least one anti-TNF antibody compositions include albumin, protamine, or the like. Typical carbohydrates useful in formulating at least one anti-TNF antibody include sucrose, mannitol, lactose, trehalose, glucose, or the like. The at least one anti-TNF antibody formulation can also include a surfactant, which can reduce or prevent surface-induced aggregation of the at least one anti-TNF antibody caused by atomization of the solution in forming an aerosol. Various conventional surfactants can be employed, such as polyoxyethylene fatty acid esters and alcohols, and polyoxyethylene sorbital fatty acid esters. Amounts will generally range between 0.001 and 4% by weight of the formulation. Especially preferred surfactants for purposes of this invention are polyoxyethylene sorbitan mono-oleate, polysorbate 80, polysorbate 20, or the like. Additional agents known in the art for formulation of a protein such as antibody protein can also be included in the formulation.

**Administration of TNF antibody compositions By A Metered Dose Inhaler**

In a metered dose inhaler (MDI), a propellant, at least one anti-TNF antibody, and any

excipients or other additives are contained in a canister as a mixture including a liquefied compressed gas. Actuation of the metering valve releases the mixture as an aerosol, preferably containing particles in the size range of less than about 10 µm, preferably about 1 µm to about 5 µm, and most preferably about 2 µm to about 3 µm. The desired aerosol particle size can be obtained by employing 5 a formulation of antibody composition produced by various methods known to those of skill in the art, including jet-milling, spray drying, critical point condensation, or the like. Preferred metered dose inhalers include those manufactured by 3M or Glaxo and employing a hydrofluorocarbon propellant.

Formulations of at least one anti-TNF antibody for use with a metered-dose inhaler device 10 will generally include a finely divided powder containing at least one anti-TNF antibody as a suspension in a non-aqueous medium, for example, suspended in a propellant with the aid of a surfactant. The propellant can be any conventional material employed for this purpose, such as chlorofluorocarbon, a hydrochlorofluorocarbon, a hydrofluorocarbon, or a hydrocarbon, including trichlorofluoromethane, dichlorodifluoromethane, dichlorotetrafluoroethanol and 1,1,1,2-tetrafluoroethane, HFA-134a (hydrofluroalkane-134a), HFA-227 (hydrofluroalkane-227), or the like. 15 Preferably the propellant is a hydrofluorocarbon. The surfactant can be chosen to stabilize the at least one anti-TNF antibody as a suspension in the propellant, to protect the active agent against chemical degradation, and the like. Suitable surfactants include sorbitan trioleate, soya lecithin, oleic acid, or the like. In some cases solution aerosols are preferred using solvents such as ethanol. 20 Additional agents known in the art for formulation of a protein such as protein can also be included in the formulation.

One of ordinary skill in the art will recognize that the methods of the current invention can be achieved by pulmonary administration of at least one anti-TNF antibody compositions via devices not described herein.

#### 25 **Oral Formulations and Administration**

Formulations for oral rely on the co-administration of adjuvants (e.g., resorcinols and nonionic surfactants such as polyoxyethylene oleyl ether and n-hexadecylpolyethylene ether) to increase artificially the permeability of the intestinal walls, as well as the co-administration of enzymatic inhibitors (e.g., pancreatic trypsin inhibitors, diisopropylfluorophosphate (DFF) and 30 trasylo) to inhibit enzymatic degradation. Formulations for delivery of hydrophilic agents including proteins and antibodies and a combination of at least two surfactants intended for oral, buccal, mucosal, nasal, pulmonary, vaginal transmembrane, or rectal administration are taught in U.S. 6,309,663. The active constituent compound of the solid-type dosage form for oral

administration can be mixed with at least one additive, including sucrose, lactose, cellulose, mannitol, trehalose, raffinose, maltitol, dextran, starches, agar, arginates, chitins, chitosans, pectins, gum tragacanth, gum arabic, gelatin, collagen, casein, albumin, synthetic or semisynthetic polymer, and glyceride. These dosage forms can also contain other type(s) of additives, e.g., inactive diluting agent, lubricant such as magnesium stearate, paraben, preserving agent such as sorbic acid, ascorbic acid, .alpha.-tocopherol, antioxidant such as cysteine, disintegrator, binder, thickener, buffering agent, sweetening agent, flavoring agent, perfuming agent, etc.

Tablets and pills can be further processed into enteric-coated preparations. The liquid preparations for oral administration include emulsion, syrup, elixir, suspension and solution preparations allowable for medical use. These preparations can contain inactive diluting agents ordinarily used in said field, e.g., water. Liposomes have also been described as drug delivery systems for insulin and heparin (U.S. Pat. No. 4,239,754). More recently, microspheres of artificial polymers of mixed amino acids (proteinoids) have been used to deliver pharmaceuticals (U.S. Pat. No. 4,925,673). Furthermore, carrier compounds described in U.S. Pat. No. 5,879,681 and U.S. Pat. No. 5,5,871,753 are used to deliver biologically active agents orally are known in the art.

#### Mucosal Formulations and Administration

A formulation for orally administering a bioactive agent encapsulated in one or more biocompatible polymer or copolymer excipients, preferably a biodegradable polymer or copolymer, affording microcapsules which due to the proper size of the resultant microcapsules results in the agent reaching and being taken up by the folliculi lymphatic aggregati, otherwise known as the "Peyer's patch," or "GALT" of the animal without loss of effectiveness due to the agent having passed through the gastrointestinal tract. Similar folliculi lymphatic aggregati can be found in the bronchial tubes (BALT) and the large intestine. The above-described tissues are referred to in general as mucosally associated lymphoreticular tissues (MALT). For absorption through mucosal surfaces, compositions and methods of administering at least one anti-TNF antibody include an emulsion comprising a plurality of submicron particles, a mucoadhesive macromolecule, a bioactive peptide, and an aqueous continuous phase, which promotes absorption through mucosal surfaces by achieving mucoadhesion of the emulsion particles (U.S. Pat. Nos. 5,514,670). Mucous surfaces suitable for application of the emulsions of the present invention can include corneal, conjunctival, buccal, sublingual, nasal, vaginal, pulmonary, stomachic, intestinal, and rectal routes of administration. Formulations for vaginal or rectal administration, e.g. suppositories, can contain as excipients, for example, polyalkyleneglycols, vaseline, cocoa butter, and the like. Formulations for intranasal administration can be solid and contain as excipients, for example, lactose or can be aqueous or oily

solutions of nasal drops. For buccal administration excipients include sugars, calcium stearate, magnesium stearate, pregelatinated starch, and the like (U.S. Pat. Nos. 5,849,695).

#### **Transdermal Formulations and Administration**

For transdermal administration, the at least one anti-TNF antibody is encapsulated in a delivery device such as a liposome or polymeric nanoparticles, microparticle, microcapsule, or microspheres (referred to collectively as microparticles unless otherwise stated). A number of suitable devices are known, including microparticles made of synthetic polymers such as polyhydroxy acids such as polylactic acid, polyglycolic acid and copolymers thereof, polyorthoesters, polyanhydrides, and polyphosphazenes, and natural polymers such as collagen, polyamino acids, albumin and other proteins, alginate and other polysaccharides, and combinations thereof (U.S. Pat. Nos. 5,814,599).

#### **Prolonged Administration and Formulations**

It can be sometimes desirable to deliver the compounds of the present invention to the subject over prolonged periods of time, for example, for periods of one week to one year from a single administration. Various slow release, depot or implant dosage forms can be utilized. For example, a dosage form can contain a pharmaceutically acceptable non-toxic salt of the compounds that has a low degree of solubility in body fluids, for example, (a) an acid addition salt with a polybasic acid such as phosphoric acid, sulfuric acid, citric acid, tartaric acid, tannic acid, pamoic acid, alginic acid, polyglutamic acid, naphthalene mono- or di-sulfonic acids, polygalacturonic acid, and the like; (b) a salt with a polyvalent metal cation such as zinc, calcium, bismuth, barium, magnesium, aluminum, copper, cobalt, nickel, cadmium and the like, or with an organic cation formed from e.g., N,N'-dibenzyl-ethylenediamine or ethylenediamine; or (c) combinations of (a) and (b) e.g. a zinc tannate salt. Additionally, the compounds of the present invention or, preferably, a relatively insoluble salt such as those just described, can be formulated in a gel, for example, an aluminum monostearate gel with, e.g. sesame oil, suitable for injection. Particularly preferred salts are zinc salts, zinc tannate salts, pamoate salts, and the like. Another type of slow release depot formulation for injection would contain the compound or salt dispersed for encapsulated in a slow degrading, non-toxic, non-antigenic polymer such as a polylactic acid/polyglycolic acid polymer for example as described in U.S. Pat. No. 3,773,919. The compounds or, preferably, relatively insoluble salts such as those described above can also be formulated in cholesterol matrix silastic pellets, particularly for use in animals. Additional slow release, depot or implant formulations, e.g. gas or liquid liposomes are known in the literature (U.S. Pat. Nos. 5,770,222 and "Sustained and Controlled Release Drug Delivery Systems", J. R. Robinson ed., Marcel Dekker, Inc., N.Y., 1978).

Having generally described the invention, the same will be more readily understood by reference to the following examples, which are provided by way of illustration and are not intended as limiting.

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#### **Example 1: Cloning and Expression of TNF antibody in Mammalian Cells**

A typical mammalian expression vector contains at least one promoter element, which mediates the initiation of transcription of mRNA, the antibody coding sequence, and signals required for the termination of transcription and polyadenylation of the transcript. Additional elements include enhancers, Kozak sequences and intervening sequences flanked by donor and acceptor sites for RNA splicing. Highly efficient transcription can be achieved with the early and late promoters from SV40, the long terminal repeats (LTRS) from Retroviruses, e.g., RSV, HTLV1, HIV1 and the early promoter of the cytomegalovirus (CMV). However, cellular elements can also be used (e.g., the human actin promoter). Suitable expression vectors for use in practicing the present invention include, for example, vectors such as pIRES1neo, pRetro-Off, pRetro-On, PLXSN, or pLNCX (Clonetech Labs, Palo Alto, CA), pcDNA3.1 (+/-), pcDNA/Zeo (+/-) or pcDNA3.1/Hygro (+/-) (Invitrogen), PSVL and PMSG (Pharmacia, Uppsala, Sweden), pRSVcat (ATCC 37152), pSV2dhfr (ATCC 37146) and pBC12MI (ATCC 67109). Mammalian host cells that could be used include human Hela 293, H9 and Jurkat cells, mouse NIH3T3 and C127 cells, Cos 1, Cos 7 and CV 1, quail QC1-3 cells, mouse L cells and Chinese hamster ovary (CHO) cells.

Alternatively, the gene can be expressed in stable cell lines that contain the gene integrated into a chromosome. The co-transfection with a selectable marker such as dhfr, gpt, neomycin, or hygromycin allows the identification and isolation of the transfected cells.

The transfected gene can also be amplified to express large amounts of the encoded antibody. The DHFR (dihydrofolate reductase) marker is useful to develop cell lines that carry several hundred or even several thousand copies of the gene of interest. Another useful selection marker is the enzyme glutamine synthase (GS) (Murphy, et al., Biochem. J. 227:277-279 (1991); Bebbington, et al., Bio/Technology 10:169-175 (1992)). Using these markers, the mammalian cells are grown in selective medium and the cells with the highest resistance are selected. These cell lines contain the amplified gene(s) integrated into a chromosome. Chinese hamster ovary (CHO) and NSO cells are often used for the production of antibodies.

The expression vectors pC1 and pC4 contain the strong promoter (LTR) of the Rous Sarcoma Virus (Cullen, et al., Molec. Cell. Biol. 5:438-447 (1985)) plus a fragment of the CMV-enhancer

(Boshart, et al., Cell 41:521-530 (1985)). Multiple cloning sites, e.g., with the restriction enzyme cleavage sites BamHI, XbaI and Asp718, facilitate the cloning of the gene of interest. The vectors contain in addition the 3' intron, the polyadenylation and termination signal of the rat preproinsulin gene.

## 5 Cloning and Expression in CHO Cells

The vector pC4 is used for the expression of TNF antibody. Plasmid pC4 is a derivative of the plasmid pSV2-dhfr (ATCC Accession No. 37146). The plasmid contains the mouse DHFR gene under control of the SV40 early promoter. Chinese hamster ovary- or other cells lacking dihydrofolate activity that are transfected with these plasmids can be selected by growing the cells in a selective medium (e.g., alpha minus MEM, Life Technologies, Gaithersburg, MD) supplemented with the chemotherapeutic agent methotrexate. The amplification of the DHFR genes in cells 10 resistant to methotrexate (MTX) has been well documented (see, e.g., F. W. Alt, et al., J. Biol. Chem. 253:1357-1370 (1978); J. L. Hamlin and C. Ma, Biochem. et Biophys. Acta 1097:107-143 (1990); and M. J. Page and M. A. Sydenham, Biotechnology 9:64-68 (1991)). Cells grown in increasing 15 concentrations of MTX develop resistance to the drug by overproducing the target enzyme, DHFR, as a result of amplification of the DHFR gene. If a second gene is linked to the DHFR gene, it is usually co-amplified and over-expressed. It is known in the art that this approach can be used to develop cell lines carrying more than 1,000 copies of the amplified gene(s). Subsequently, when the methotrexate is withdrawn, cell lines are obtained that contain the amplified gene integrated into one or more 20 chromosome(s) of the host cell.

Plasmid pC4 contains for expressing the gene of interest the strong promoter of the long terminal repeat (LTR) of the Rous Sarcoma Virus (Cullen, et al., Molec. Cell. Biol. 5:438-447 (1985)) plus a fragment isolated from the enhancer of the immediate early gene of human cytomegalovirus (CMV) (Boshart, et al., Cell 41:521-530 (1985)). Downstream of the promoter are 25 BamHI, XbaI, and Asp718 restriction enzyme cleavage sites that allow integration of the genes. Behind these cloning sites the plasmid contains the 3' intron and polyadenylation site of the rat preproinsulin gene. Other high efficiency promoters can also be used for the expression, e.g., the human b-actin promoter, the SV40 early or late promoters or the long terminal repeats from other retroviruses, e.g., HIV and HTLV. Clontech's Tet-Off and Tet-On gene expression systems and 30 similar systems can be used to express the TNF in a regulated way in mammalian cells (M. Gossen, and H. Bujard, Proc. Natl. Acad. Sci. USA 89: 5547-5551 (1992)). For the polyadenylation of the mRNA other signals, e.g., from the human growth hormone or globin genes can be used as well. Stable cell lines carrying a gene of interest integrated into the chromosomes can also be selected upon

co-transfection with a selectable marker such as gpt, G418 or hygromycin. It is advantageous to use more than one selectable marker in the beginning, e.g., G418 plus methotrexate.

The plasmid pC4 is digested with restriction enzymes and then dephosphorylated using calf intestinal phosphatase by procedures known in the art. The vector is then isolated from a 1% agarose gel.  
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The DNA sequence encoding the HC and/or LC variable regions of the TNF antibody is used, e.g., as presented in SEQ ID NOS:7, 8, 15 or 16, corresponding to HC and LC variable regions of a TNF antibody of the present invention, according to known method steps. Isolated nucleic acid encoding a suitable human constant region (i.e., HC and LC regions) is also used in this construct.

10 The isolated variable and constant region encoding DNA and the dephosphorylated vector are then ligated with T4 DNA ligase. E. coli HB101 or XL-1 Blue cells are then transformed and bacteria are identified that contain the fragment inserted into plasmid pC4 using, for instance, restriction enzyme analysis.

Chinese hamster ovary (CHO) cells lacking an active DHFR gene are used for transfection. 5  
15 μg of the expression plasmid pC4 is cotransfected with 0.5 μg of the plasmid pSV2-neo using lipofectin. The plasmid pSV2neo contains a dominant selectable marker, the neo gene from Tn5 encoding an enzyme that confers resistance to a group of antibiotics including G418. The cells are seeded in alpha minus MEM supplemented with 1 μg /ml G418. After 2 days, the cells are trypsinized and seeded in hybridoma cloning plates (Greiner, Germany) in alpha minus MEM  
20 supplemented with 10, 25, or 50 ng/ml of methotrexate plus 1 μg /ml G418. After about 10-14 days single clones are trypsinized and then seeded in 6-well petri dishes or 10 ml flasks using different concentrations of methotrexate (50 nM, 100 nM, 200 nM, 400 nM, 800 nM). Clones growing at the highest concentrations of methotrexate are then transferred to new 6-well plates containing even higher concentrations of methotrexate (1 mM, 2 mM, 5 mM, 10 mM, 20 mM). The same procedure  
25 is repeated until clones are obtained that grow at a concentration of 100 - 200 mM. Expression of the desired gene product is analyzed, for instance, by SDS-PAGE and Western blot or by reverse phase HPLC analysis.

#### Binding Kinetics of Human Anti-Human TNF antibodies

ELISA analysis confirms that purified antibody from these host cells bind TNF in a concentration-dependent manner. In this case, the avidity of the antibody for its cognate antigen (epitope) is measured. Quantitative binding constants are obtained using BIACore analysis of the human antibodies and reveals that several of the human monoclonal antibodies are very high affinity with  $K_D$  in the range of  $1 \times 10^{-9}$  to  $9 \times 10^{-12}$ .

### Conclusions

Human TNF reactive IgG monoclonal antibodies of the invention are generated. The human anti-TNF antibodies are further characterized. Several of generated antibodies have affinity constants between  $1 \times 10^9$  and  $9 \times 10^{12}$ . The unexpectedly high affinities of these fully human monoclonal 5 antibodies make them suitable for therapeutic applications in TNF-dependent diseases, pathologies or related conditions.

### EXAMPLE 2: Ability Of TNF $\alpha$ Specific Monoclonal Antibodies (MABs) To Inhibit TNF $\alpha$ Mediated Murine Tumor Cell Killing

10 Cell killing assays were used to examine the ability of the 9C1.1, 5E1.4, 5D1, 1A2, 9C1.20, 9E6.6, 9E7.1, 10D5, and 11E11.6 to inhibit TNF $\alpha$  mediated tumor cell killing. To initiate cell killing assays murine fibrosarcoma WEHI 164 cells were grown in High Glucose DMEM (Mediatech, Inc., Herndon, VA, USA) containing 10% FBS (Hyclone, Logan, Utah), penicillin-streptomycin and 15  $5.5 \times 10^{-5}$  M 2-mercaptoethanol (GIBCO BRL Invitrogen, Corp., Carlsbad, CA, USA) in a humidified 37°C incubator containing 5% CO<sub>2</sub>. WEHI cells were then harvested by trypsinization and seeded in High Glucose DMEM containing 10% FBS at 50,000 cells/well in a 96-well tissue culture plate and treated with actinomycin-D at a final concentration of 2  $\mu$ g/ml. Plates were then incubated at 37°C in a 5% CO<sub>2</sub> atmosphere for 4 hours. The MABs indicated in FIG. 1A-F were preincubated with human 20 TNF $\alpha$  for 30 minutes and this mixture was added to the WEHI 164 cell media. Plates were then incubated overnight at 37°C, in a 5% CO<sub>2</sub> atmosphere. The final TNF $\alpha$  concentration in the cell media was either 0.2 ng/ml (FIG 1A, C, E) or 0.4 ng/ml (FIG 1B, D, F). The final MAB concentrations in the cell media were as indicated in FIG. 1A-F. Negative controls included cell media alone or cell media with 0.2 ng/ml (FIG. 1A, C, E) or 0.4 ng/ml (FIG. 1B, D, F) TNF $\alpha$ . In 25 FIG. 1E-F the negative control antibody was the CD152 specific ANC152.2/8H5 MAB (ANCELL Corp., Bayport, MN, USA) which was added to the cell media at the final concentrations indicated in FIG. 1E-F. The positive control antibody in FIG. 1E-F was the TNF $\alpha$  specific, human J1D9 MAB (ANCELL Corp., Bayport, MN, USA) which was also added to the final concentrations indicated in FIG. 1E-F. Cell viability was determined with the CELLTITER 96 Aqueous One Solution Cell Proliferation Assay system (Promega, Madison, WI, USA) as directed by the manufacturer and the 30 final absorbance at 492 nm was used to determine cell viability.

As shown in FIG. 1A and FIG 1B, increasing concentrations of the 9C1.1 MAB inhibited WEHI 164 cell killing mediated by 0.2 ng/ml (FIG. 1A) or 0.4 ng/ml (FIG. 1B) TNF $\alpha$ . Additionally, the 9C1.1 MAB inhibited TNF $\alpha$  mediated cell killing at much lower antibody concentrations than the

5E1.4, 5D1 and 1A2 MABs (FIG. 1A or 1B).

FIG. 1C and FIG. 1D reveal that increasing concentrations of the 9C1.20, 9E6.6, and 9E7.1 MABs inhibit WEHI 164 cell killing mediated by 0.2 ng/ml (FIG. 1C) or 0.4 ng/ml (FIG. 1D) TNF $\alpha$ .

FIG. 1E and FIG. 1F show the effects of increasing concentrations of the 10D5, and 11E11.6 MABs on WEHI 164 cell killing mediated by 0.2 ng/ml (FIG. 1E) or 0.4 ng/ml (FIG. 1F) TNF $\alpha$ . As seen in FIG. 1E and FIG. 1F, the 10D5 and 11E11.6 MABs inhibited TNF $\alpha$  mediated cell killing at much lower antibody concentrations than the J1D9 positive control MAB.

**EXAMPLE 3: Ability Of TNF $\alpha$  Specific MABs To Inhibit TNF $\alpha$  Mediated Human Tumor Cell  
10 Killing**

A cell killing assay was also used to examine the ability of the 9E6.6, 9E7.1, 10D5.1, 11E11.6, 9C1.20, 9C1.1, 5E1.4 and 1A2.8 MABs to inhibit TNF $\alpha$  mediated tumor cell killing. To initiate the cell killing assay, human rhabdomyosarcoma KYM-1D4 cells were seeded at 50,000 cells/well in a 96-well tissue culture plate, treated with actinomycin-D at a final concentration of 2  $\mu$ g/ml and incubated at 37°C in a 5% CO<sub>2</sub> atmosphere for 4 hours. The MABs indicated in FIG. 2 were then preincubated with human TNF $\alpha$  for 30 minutes. Next the KYM-1D4 cells were incubated overnight at 37°C in a 5% CO<sub>2</sub> atmosphere with each MAB and human TNF $\alpha$  mixture. The final TNF $\alpha$  concentration in the cell media was 0.2 ng/ml (FIG 2). The final MAB concentrations were as indicated in FIG. 2. The positive control antibody consisted of the TNF $\alpha$  specific rTNV148B MAB 20 at the concentrations indicated in FIG. 2. Cell viability was determined by adding methylthiazole tetrazolium (MTT) (Sigma-Aldrich Fine Chemicals, St. Louis, MO, USA) to the culture media to a final concentration of 0.5 mg/ml, incubating for 3 hr at 37°C in 5% CO<sub>2</sub> atmosphere, removing the media, adding dimethylsulfoxide (DMSO) (Sigma-Aldrich Fine Chemicals, St. Louis, MO, USA) and measuring A<sub>550-650nm</sub>.

The effects of increasing concentrations of the 9E6.6, 9E7.1, 10D5.1, 11E11.6, 9C1.20, 9C1.1, 5E1.4 and 1A2.8 MABs on human rhabdomyosarcoma KYM-1D4 cell killing mediated by 0.2 ng/ml TNF $\alpha$  can be seen in FIG. 2. Importantly, the 9E6.6, 9E7.1, 10D5.1, 11E11.6, 9C1.20, and 9C1.1 MABs inhibited TNF $\alpha$  mediated cell killing at much lower antibody concentrations than the 5E1.4 and 1A2.8 MABs (FIG. 2).

**EXAMPLE 4: Sequence analysis of RNAs Encoding TNF $\alpha$  Specific MABs**

Sequence analysis of the RNAs encoding the TNF $\alpha$  specific 9C1.1, 9C1.20, 9E6.6, 9E7.1, 10D1.5, and 11E11.6 MABS was undertaken to further characterize these MABS. To begin this analysis total cellular RNA from the hybridomas producing these MABs was isolated using standard methods. Next, cDNAs encoding the variable regions of the heavy chains (HC) and light chains (LC) of the antibodies encoded by these hybridomas were amplified by the reverse transcriptase polymerase chain reaction (RT-PCR). Determination of the variable region sequences confirmed that the antibodies were of human sequence. Two human light chain sequences, 9C1LC and 9E7LC, were amplified from each clone (FIG. 3). One HC variable region sequence was amplified from the total RNA of the 9C1.1, 9C1.20, 9E6.6, 9E7.1, 10D1.5, and 11E11.6 hybridomas. This HC sequence was identical in all six hybridoma cell lines and was named 9C1HC (FIG. 4).

Total cellular RNA was also prepared by the above method from the subcloned 9C1.1.14, 9C1.1.3, 9C1.7.5, 9C1.7.12 and 9C1.9.8 hybridomas. RT-PCR of the method described above was then used to amplify cDNAs encoding the HC and LC variable regions from the total RNA prepared from these subcloned hybridomas. Again, two human light chain sequences, 9C1LC and 9E7LC, were amplified from each clone (FIG. 3). However, a distinct HC variable region sequence designated 9C1AHC was amplified (FIG. 4). CLUSTAL alignment (Higgins, D. G. et al. Using CLUSTAL for multiple sequence alignments. Methods Enzymol., 266, 383-402 (1996)) of the 9C1AHC and 9C1HC sequences revealed that the 9C1AHC sequence differs from 9C1HC by only three nucleotides (FIG. 4). However, this three nucleotide difference between the 9C1AHC and 9C1HC sequences only translates into a single amino acid substitution as two of the nucleotide changes are silent. The result of these sequence differences is that the 9C1HC amino acid sequence has a glutamic acid residue at position 50, while 9C1AHC has a lysine residue at this position. This substitution changes the first amino acid residue of complementarity determining region 2 (CDR2) in the HC variable region.

**EXAMPLE 5: Preparation of TNF $\alpha$  Specific Recombinant Antibodies**

cDNAs encoding the 9C1HC and 9C1AHC heavy chain sequences and the 9C1LC and 9E7LC light chain sequences were cloned into plasmid vectors. To produce stably transfected cell lines, plasmids encoding either 9C1HC or 9C1AHC were transfected in combination with the plasmids encoding 9C1LC and 9E7LC into SP2/0 myeloma cells. Stably transfected, monoclonal cell lines were isolated and screened by an ELISA assay for TNF $\alpha$  specific MAB expression.

**TNF $\alpha$  Specific ELISAs**

The TNF $\alpha$  expression screening ELISA was initiated by coating 96 well NUNC MAXISORP plates with 100  $\mu$ l of 1  $\mu$ g/ml TNF $\alpha$ . The plates were washed with 0.15 M PBS containing 0.02% TWEEN 20 (Sigma-Aldrich Fine Chemicals, St. Louis, MO, USA). Culture media from stably transfected, monoclonal cell lines was serially diluted and placed in the TNF $\alpha$  coated, 96 well plates. Plates were incubated for 1 hr at room temperature and washed three times with PBS. The plates were then incubated with AP conjugated goat anti-human IgG antibody for 30 min at room temperature and washed again. pNPPsubstrate solution was then added and the plates were incubated at room temperature until color developed. To stop reactions 3 M NaOH was added to each well.

Final absorbance at 405 nm was then measured and compared to a calibration plot generated with human TNF $\alpha$  specific IgG1 antibody of known concentration.

Two recombinant antibodies r9C1 (9C1HC/9C1LC) and r9C1A (9C1AHC/9C1LC) capable of binding to human TNF $\alpha$  were identified by the screening ELISA protocol described above. Recombinant antibodies containing the 9E7LC MAB were unable to bind human TNF $\alpha$  under the conditions of this screening ELISA protocol.

**EXAMPLE 6: Ability Of Recombinant, TNF $\alpha$  Specific MABs To Inhibit TNF $\alpha$  Mediated WEHI 164 Tumor Cell Killing**

Cell killing assays were used to examine the ability of the r9C1 (9C1HC/9C1LC) and r9C1A (9C1AHC/9C1LC) MABs to inhibit TNF $\alpha$  mediated tumor cell killing. To initiate cell killing assays murine fibrosarcoma WEHI 164 cells were seeded at 50,000 cells/well in a 96-well tissue culture plate and treated with actinomycin-D at a final concentration of 2  $\mu$ g/ml. Plates were then incubated at 37°C in a 5% CO<sub>2</sub> atmosphere for 4 hours. The MABs indicated in FIG. 5 were preincubated with human TNF $\alpha$  for 30 minutes and this mixture was added to the WEHI 164 cell media. Plates were then incubated overnight at 37°C, in a 5% CO<sub>2</sub> atmosphere. The final TNF $\alpha$  concentration in the cell media was 0.2 ng/ml. The final MAB concentrations in the cell media were as indicated in FIG. 5. The positive control antibody in FIG. 5 was the highly potent, human TNF $\alpha$  specific rTNV148B MAB which was added to the final concentrations indicated in FIG. 5. Cell viability was determined by adding MTT to the culture media to a final concentration of 0.5 mg/ml, incubating for 3 hours at 37°C in 5%CO<sub>2</sub> atmosphere, removing the media adding DMSO and measuring absorbance at 550 nm.

As seen in FIG. 5 the r9C1A MAB is substantially more potent than the r9C1 MAB and approximately 5 fold more potent than the positive control rTNV148B MAB.

**EXAMPLE 7: Ability Of Recombinant, TNF $\alpha$  Specific MABs To Inhibit TNF $\alpha$  Binding to**

**5 Recombinant TNF $\alpha$  Receptor**

Binding assays were performed to examine the ability of r9C1 and r9C1A to inhibit TNF $\alpha$  binding to recombinant TNF $\alpha$  receptor. The recombinant TNF $\alpha$  receptor used was the P55-sf2 protein. P55-sf2 consists of the TNF $\alpha$  receptor fused in frame to an antibody Fc fusion protein. Briefly, 0.5  $\mu$ g/ml purified p55-sf2 in 100  $\mu$ l was coated onto 96 well NUNC MAXISORB ELISA plates. Next hybridoma cell media containing known amounts of the MABS indicated in FIG. 6 was preincubated with 5 ng/ml of  $^{125}$ I labeled TNF $\alpha$ .  $^{125}$ I labeled TNF $\alpha$  and MAB mixtures were then transferred to the 96 well ELISA plates which had been coated with p55-sf2. The identity and final concentrations of MABs were as indicated in FIG. 6. Negative controls included cSF25 an unrelated antibody control,  $^{125}$ I labeled TNF $\alpha$  alone and the negative control, isotype matched 9C1AHC/9E7LC MAB. The positive control was the rTNV148B MAB which is TNF $\alpha$  specific. Plates were incubated for 2 hr at room temperature and washed to remove unbound  $^{125}$ I labeled TNF $\alpha$ . The amount of TNF $\alpha$  bound in the presence of varying amounts of antibody was quantified using a gamma radiation counter.

As seen in FIG. 6 the r9C1A MAB inhibited  $^{125}$ I labeled TNF $\alpha$  binding to the recombinant P55-sf2 TNF $\alpha$  receptor. In contrast, the r9C1 MAB was unable to prevent the binding of TNF $\alpha$  to the P55-sf2 TNF $\alpha$  receptor as shown in FIG. 6. Additionally, the r9C1A MAB inhibits TNF $\alpha$  binding at an approximately 5 fold lower concentration than the positive control rTNV148B MAB (FIG. 6); this is consistent with the results of the WEHI 164 tumor cell killing experiment of FIG. 5.

25 It will be clear that the invention can be practiced otherwise than as particularly described in the foregoing description and examples.

Numerous modifications and variations of the present invention are possible in light of the above teachings and, therefore, are within the scope of the appended claims.

**WHAT IS CLAIMED IS:**

- 1 . At least one isolated mammalian ANTI-TNF antibody, comprising at least one variable region comprising at least one heavy chain and at least one light chain of SEQ ID NOS:7, 8, 15, or 16.
- 5 2 . At least one isolated mammalian anti-TNF antibody, comprising either (i) at least two of the heavy chain complementarity determining regions (CDR) amino acid sequences of at least one of SEQ ID NOS:1, 2, 3, 9, 10 or 11; or (ii) at least two of the light chain CDR amino acids sequences of at least one of SEQ ID NOS:4, 5, 6, 12, 13, or 14.
- 10 3 . At least one isolated mammalian anti-TNF antibody, comprising at least one heavy chain or light chain CDR having the amino acid sequence of at least one of SEQ ID NOS:1, 2, 3, 4, 5, 6, 9, 10, 11, 12, 13, or 14.
- 15 4 . At least one isolated mammalian anti-TNF antibody that binds to the same region of a anti-TNF polypeptide as an antibody comprising at least one heavy chain or light chain CDR having the amino acid sequence of at least one of SEQ ID NOS: 1, 2, 3, 4, 5, 6, 9, 10, 11, 12, 13, 14.
- 20 5 . At least one isolated mammalian anti-TNF antibody, comprising at least one human CDR, wherein said antibody specifically inhibits the binding of at least one anti-TNF antibody according to any of claim 1-4.
- 25 6 . An anti-TNF antibody according to any of claims 1-6, wherein said antibody binds anti-TNF with an affinity of at least one selected from at least  $10^9$  M, at least  $10^{10}$  M, at least  $10^{11}$  M, or at least  $10^{12}$  M.
- 7 . An anti-TNF antibody according to to any of claims 1-6, wherein said antibody substantially modulates at least one activity of at least one anti-TNF polypeptide.
- 30 8 . An isolated nucleic acid encoding at least one isolated mammalian anti-TNF antibody according to any of claims 1-6 and having at least one human CDR, wherein said antibody specifically inhibits the binding of at least one anti-TNF antibody according to any of claim 1-4.
- 9 . An isolated nucleic acid vector comprising an

isolated nucleic acid according to claim 9.

10. A prokaryotic or eukaryotic host cell comprising an isolated nucleic acid according to claim 9.

5

A host cell according to claim 10, wherein said host cell is at least one selected from COS-1, COS-7, HEK293, BHK21, CHO, BSC-1, Hep G2, 653, SP2/0, 293, HeLa, myeloma, or lymphoma cells, or any derivative, immortalized or transformed cell thereof.

10

A method for producing at least one anti-TNF antibody, comprising translating a nucleic acid according to claim 9 under conditions in vitro, in vivo or in situ, such that the anti-TNF antibody is expressed in detectable or recoverable amounts.

15

A composition comprising at least one isolated mammalian anti-TNF antibody according to any of claims 1-6 having at least one human CDR, wherein said antibody specifically binds at least one epitope comprising at least 1-3, to the entire amino acid sequence of SEQ ID NO:17, and at least one pharmaceutically acceptable carrier or diluent.

20

A composition according to claim 13, further comprising at least one at least one compound or polypeptide selected from at least one of a detectable label or reporter, a TNF antagonist, an anti-infective drug, a cardiovascular (CV) system drug, a central nervous system (CNS) drug, an autonomic nervous system (ANS) drug, a respiratory tract drug, a gastrointestinal (GI) tract drug, a hormonal drug, a drug for fluid or electrolyte balance, a hematologic drug, an antineoplastic, an immunomodulation drug, an ophthalmic, otic or nasal drug, a topical drug, a nutritional drug, a cytokine, or a cytokine antagonist.

25

An anti-idiotype antibody or fragment that specifically binds at least one anti-TNF antibody according to any of claims 1-6.

30

16. A method for diagnosing or treating a anti-TNF related condition in a cell, tissue, organ or animal, comprising

(a) contacting or administering a composition comprising an effective amount of at least one antibody according to any of claims 1-6, with, or to, said cell, tissue, organ or animal.

17. A method according to claim 16, wherein said effective amount is 0.001-50 mg/kilogram of said cells, tissue, organ or animal.

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18. A method according to claim 16, wherein said contacting or said administrating is by at least one mode selected from parenteral, subcutaneous, intramuscular, intravenous, intrarticular, intrabronchial, intraabdominal, intracapsular, intracartilaginous, intracavitory, intracelial, intracelebellar, intracerebroventricular, intracolic, intracervical, intragastric, intrahepatic, intramyocardial, intraosteal, intrapelvic, intrapericardiac, intraperitoneal, intrapleural, intraprostatic, intrapulmonary, intrarectal, intrarenal, intraretinal, intraspinal, intrasynovial, intrathoracic, intrauterine, intravesical, intralesional, bolus, vaginal, rectal, buccal, sublingual, intranasal, or transdermal.

19. A method according to 16, further comprising administering, prior, concurrently or after said (a) contacting or administering, at least one composition comprising an effective amount of at least one compound or polypeptide selected from at least one of a detectable label or reporter, an anti-infective drug, a cardiovascular (CV) system drug, a central nervous system (CNS) drug, an autonomic nervous system (ANS) drug, a respiratory tract drug, a gastrointestinal (GI) tract drug, a hormonal drug, a drug for fluid or electrolyte balance, a hematologic drug, an antineoplastic, an immunomodulation drug, an ophthalmic, otic or nasal drug, a topical drug, a nutritional drug, a cytokine, or a cytokine antagonist.

20. A medical device, comprising at least one anti-TNF antibody according to any of claims 1-6, wherein said device is suitable to contacting or administerting said at least one anti-TNF antibody by at least one mode selected from parenteral, subcutaneous, intramuscular, intravenous, intrarticular, intrabronchial, intraabdominal, intracapsular, intracartilaginous, intracavitory, intracelial, intracelebellar, intracerebroventricular, intracolic, intracervical, intragastric, intrahepatic, intramyocardial, intraosteal, intrapelvic, intrapericardiac, intraperitoneal, intrapleural, intraprostatic, intrapulmonary, intrarectal, intrarenal, intraretinal, intraspinal, intrasynovial, intrathoracic, intrauterine, intravesical, intralesional, bolus, vaginal, rectal, buccal, sublingual, intranasal, or transdermal.

21. An article of manufacture for human pharmaceutical or diagnostic use, comprising packaging material and a container

comprising a solution or a lyophilized form of at least one anti-TNF antibody according to any of claims 1-6.

22. The article of manufacture of claim 21, wherein  
said container is a component of a parenteral, subcutaneous, intramuscular,  
5 intravenous, intrarticular, intrabronchial, intraabdominal, intracapsular,  
intracartilaginous, intracavitory, intracelial, intracelebellar,  
intracerebroventricular, intracolic, intracervical, intragastric, intrahepatic,  
intramyocardial, intraosteal, intrapelvic, intrapericardiac, intraperitoneal,  
10 intrapleural, intraprostatic, intrapulmonary, intrarectal, intrarenal, intraretinal,  
intraspinal, intrasynovial, intrathoracic, intrauterine, intravesical, intralesional,  
bolus, vaginal, rectal, buccal, sublingual, intranasal, or transdermal delivery  
device or system.

23. A method for producing at least one isolated  
mammalian anti-TNF antibody according to any of claims 1-6, comprising  
15 providing a host cell or transgenic animal or transgenic plant or plant cell capable  
of expressing in recoverable amounts said antibody.

24. At least one anti-TNF antibody produced by a  
method according to claim 23.

25. Any invention described herein

20

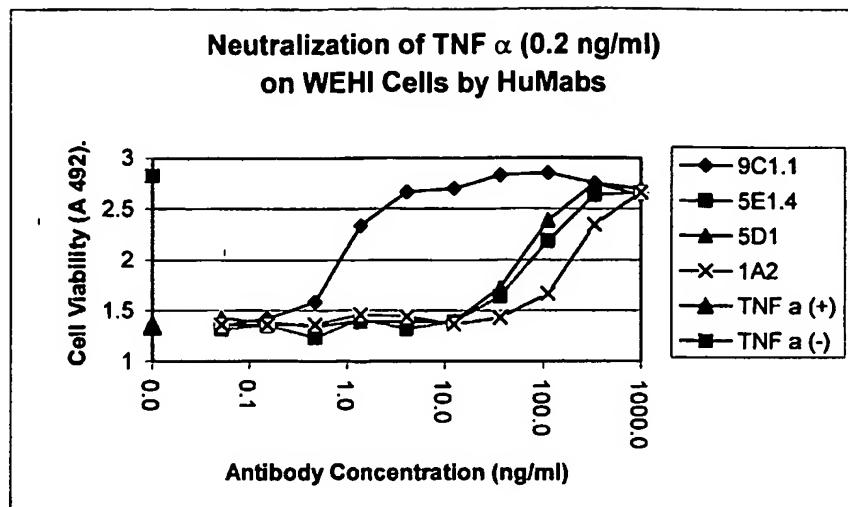


FIG. 1A

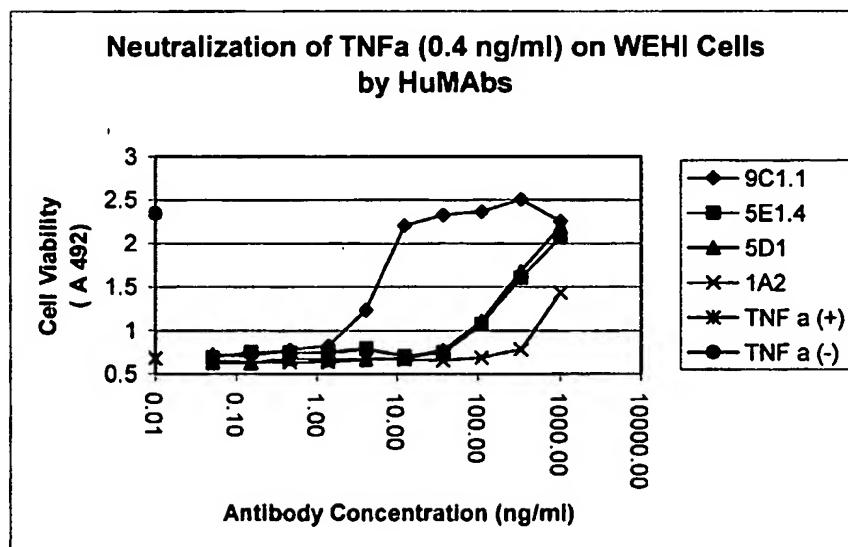


FIG. 1B

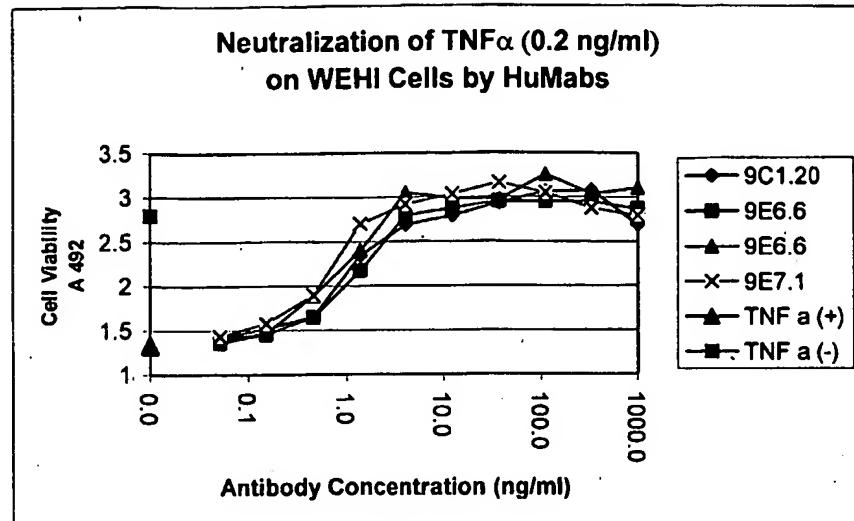


FIG. 1C

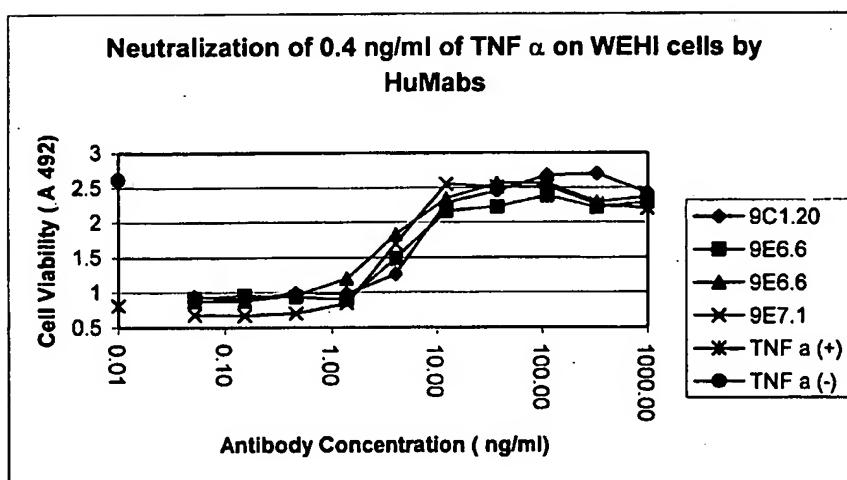


FIG. 1D

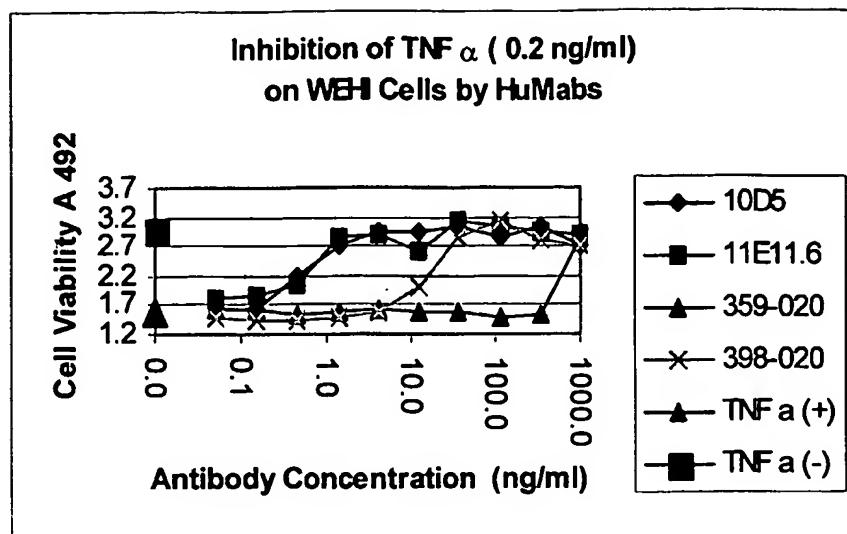


FIG. 1E

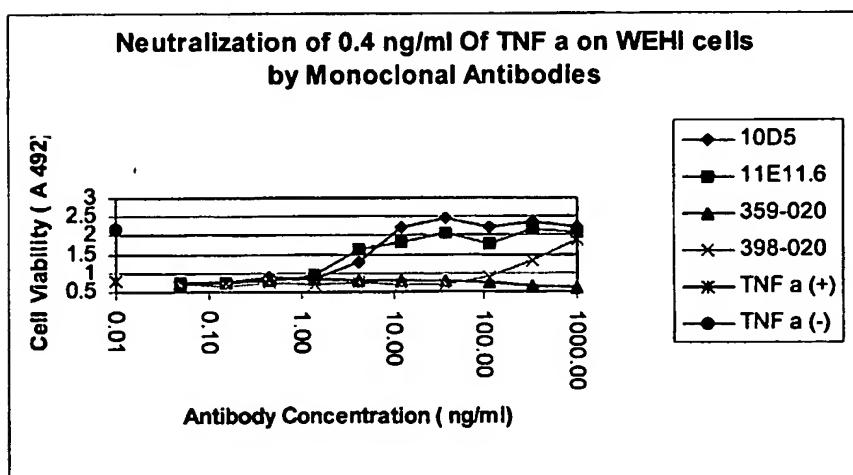


FIG. 1F

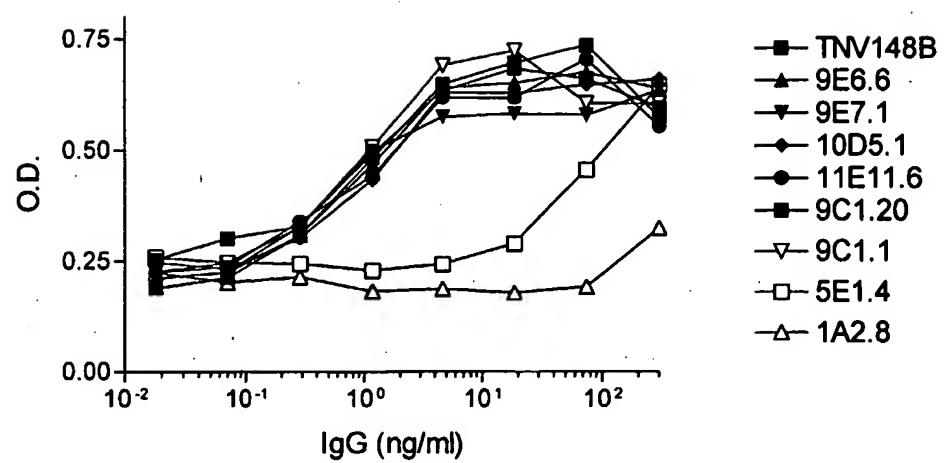


FIG. 2

**9C1ALC Light Chain Nucleotide Sequence (Variable region and J-region)**

GCCATTCCAGTTGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAAGGAGACAGAGTCACCATCACTTGCGGGCAAGTCAGGG  
CATTAGCAGTGTCTTAGCTCTGTATCAGCAGAAACCAGGGAAAGCTCTAAGCTCTGTATCTATGATGCTCTCTATTGGAAA  
GTGGGGTCCCCATCAAGGTTCAGGGCAGTGGATCTGGGACAGATTTCACTCTACCATCAGCAGCTGCAAGCTGAAGATT  
GCAACTTATTACTGTCAACAGTTAAAAGTTACCCGATCACCTTCGGCCAAGGGACACGACTGGAGATTAAACGAAC

**9C1ALC Light Chain Amino Acid Sequence (Variable region and J-region)**

Ala-Ile-Gln-Leu-Thr-Gln-Ser-Pro-Ser-Ser-Leu-Ser-Ala-Ser-Val-Gly-Asp-Arg-Val-Thr-Ile-Thr-Cys-Arg-Ala-Ser-Gln-Gly-Ile-Ser-Ser-Ala-Leu-Ala-Trp-Tyr-Gln-Gln-Lys-Pro-Gly-Lys-Ala-Pro-Lys-Leu-Leu-Ile-Tyr-Asp-Ala-Ser-Tyr-Leu-Glu-Ser-Gly-Val-Pro-Ser-Arg-Phe-Ser-Gly-Ser-Gly-Thr-Asp-Phe-Thr-Leu-Thr-Ile-Ser-Ser-Leu-Gln-Pro-Glu-Asp-Phe-Ala-Thr-Tyr-Tyr-Cys-Gln-Gln-Phe-Lys-Ser-Tyr-Pro-Ile-Thr-Phe-Gly-Gln-Gly-Thr-Arg-Leu-Glu-Ile-Lys-Arg-Thr

**9C1LC Light Chain Nucleotide Sequence (Variable region and J-region)**

GCCATTCCAGTTGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAGTCACCATCACTGGCGGGCAAGTCAGGG  
CATTAGCAGTGTCTTAGCCTGGTATCAGCAGAAACCAGGGAAAGCTCTAAGCTCTGTATCTATGATGCCCTCTATTGGAAA  
GTGGGGTCCCCTCAAGGTTCAGGCGCAGTGGACTGGGACAGATTTCCTACTCTCACCATCAGCAGCCTGCAGCCTGAAGATT  
GCAACTTATTACTGTCAACAGTTAAAGTTACCGATCACCTTCGGCCAAGGGACAGCAGTGGAGATTAAACGAAC

SC11C Light Chain Amino Acid Sequence (Variable region and J-region) Ala-Ile-Gln-

Leu-Thr-Gln-Ser-Pro-Ser-Ser-Leu-Ser-Ala-Ser-Val-Gly-Asp-Arg-Val-Thr-Ile-Thr-Cys-Arg-Ala-Ser-Gln-Gly-Ile-Ser-Ser-Ala-Leu-Ala-Trp-Tyr-Gln-Gln-Lys-Pro-Gly-Lys-Ala-Pro-Lys-Leu-Leu-Ile-Tyr-Asp-Ala-Ser-Tyr-Leu-Glu-Ser-Gly-Val-Pro-Ser-Arg-Phe-Ser-Gly-Ser-Gly-Ser-Gly-Thr-Asp-Phe-Thr-Leu-Thr-Ile-Ser-Ser-Leu-Gln-Pro-Glu-Asp-Phe-Ala-Thr-Tyr-Tyr-Cys-Gln-Gln-Phe-Lys-Ser-Tyr-Pro-Ile-Thr-Phe-Gly-Gln-Gly-Thr-Arg-Leu-Glu-Ile-Lys-Arg-Thr

**9C1AHC Heavy Chain Nucleotide Sequence (Variable region and J-region)**

CAGGTGCGACTGGTGCAGTCCTGGGCTGAGGTGAAGAAGCCTGGGTCTCGGTGAAGGTCTCCCTGAGGGCTTCTGGAGGCAC  
CTTCAGCAACTATGCTATCAGCTGGTGCACAGGCCCTGGACAAGGGCTTGAGTTAATGGGAAAGATCATCCCTATCCCTG  
GTATTACCAAACTAACGTCAGAACAGTCCAGGACAGAGTCACGATTACCGCGGACAGATCACGGACACAGCCTACATGGAGCTG  
AGCAGCCTGAGATCTGAGGACACGGCGCTATTATTGTGCGAGAGGAGGAAGCTACAGCTGGTTCGACCCCTGGGGCAGGG  
AACCCTGGTACCGCTCTCTCA

**9C1AHC Heavy Chain Amino Acid Sequence (Variable region and J-region)**

Gln-Val-Gln-Leu-Val-Gln-Ser-Gly-Ala-Glu-Val-Lys-Lys-Pro-Gly-Ser-Ser-Val-Lys-Val-Ser-Cys-Arg-Ala-Ser-Gly-Gly-Thr-Phe-Ser-Asn-Tyr-Ala-Ile-Ser-Trp-Val-Arg-Gln-Ala-Pro-Gly-Gln-Gly-Leu-Glu-Leu-Met-Gly-Lys-Ile-Ile-Pro-Ile-Leu-Gly-Ile-Thr-Asn-Tyr-Val-Gln-Lys-Phe-Gln-Asp-Arg-Val-Thr-Ile-Thr-Ala-Asp-Arg-Ser-Thr-Ser-Thr-Ala-Tyr-Met-Glu-Leu-Ser-Ser-Leu-Arg-Ser-Glu-Asp-Thr-Ala-Val-Tyr-Tyr-Cys-Ala-Arg-Gly-Gly-Ser-Tyr-Ser-Trp-Phe-Asp-Pro-Trp-Gly-Gln-Gly-Thr-Leu-Val-Thr-Val-Ser-Ser

**9C1HC Heavy Chain Nucleotide Sequence (Variable region and J-region)**

CAGGTGCAGCTGGTGCAGTCGGGCTGAGGTGAAGAAGCCTGGGTCCCTCGGTGAAGGTCTCTGCAGGGCTCTGGAGGCAC  
CTTCAGCAACTATGCCATCAGCTGGTCCGACAGGCCCTGGACAAGGGTTGAGTTAATGGGAGAGATCATCCCTATCCTTG  
GTATTACAAACTACGTACAGAAGTTCAGGACAGAGTCACGATTACGGCCAGATCCACGAGCACAGCCTACATGGAGCTG  
AGCAGCCTGAGATCTGAGGACACGGCCGTATTATTGTGCGAGAGGAGGAAGCTACAGCTGGTTCGACCCCTGGGGCCAGGG  
AACCTGGTCACCGTCTCCCTCA

**9C1HC Heavy Chain Amino Acid Sequence (Variable region and J-region)**

Gln-Val-Gln-Leu-Val-Gln-Ser-Gly-Ala-Glu-Val-Lys-Lys-Pro-Gly-Ser-Ser-Val-Lys-Val-Ser-Cys-Arg-Ala-Ser-Gly-Gly-Thr-Phe-Ser-Asn-Tyr-Ala-Ile-Ser-Trp-Val-Arg-Gln-Ala-Pro-Gly-Gln-Gly-Leu-Glu-Leu-Met-Gly-Glu-Ile-Ile-Pro-Ile-Leu-Gly-Ile-Thr-Asn-Tyr-Val-Gln-Lys-Phe-Gln-Asp-Arg-Val-Thr-Ile-Thr-Ala-Asp-Arg-Ser-Thr-Ser-Thr-Ala-Tyr-Met-Glu-Leu-Ser-Ser-Leu-Arg-Ser-Glu-Asp-Thr-Ala-Val-Tyr-Tyr-Cys-Ala-Arg-Gly-Gly-Ser-Tyr-Ser-Trp-Phe-Asp-Pro-Trp-Gly-Gln-Gly-Thr-Leu-Val-Thr-Val-Ser-Ser

FIG. 3A

**9E7LC Light Chain Nucleotide Sequence:**

ATGGAAGCCCCAGCTCAGCTCTCTTCCCTCGTACTCTGGCTCCCAGATACTTCGGGGTCGAGTCGAAGAGAAGGAGGAC  
GATGAGACCGAGGGTCTTACACCGGAGAAATTGTGTTGACACAGTCTCCAGCCACCCCTGTCTTGATGGTGGCCTTTAA  
CACAACTGTGTCAGAGGTCGGTGGGACAGAAACACTCAGGGGAAAGAGGCCACCTCTCCTGCAGGGCCAGTCAGAGTGGTAG  
CGAGGTCCCCCTTCGCGTGGGAGAGGACGTCAGTCTCAAACTCGAGCTACTTAGCTCGGTACCAACAGAAACCTGG  
CCAGGCTCCCAGGCTCCCTCGATGAATCGGACCATGGTTGCTTTGGACCGGTCCGAGGGTCCGAGGACATCTATGATGCATC  
CAACAGGGCCACTGGCATTCCACGGCTCAGTGGTAGATACGCTAGGTTGCTCCGGTGACCGTAGGGTCGGTCCAAGTC  
ACGAGTGGCTGGGACAGACTTCACCTCACCACAGCAGCTAGGCTCGTCAACCCAGACCCCTGCTGAAGTGAGAGTG  
GTAGTCGTCGGATCTCGGAGAAAGATTTGCAGTTTATACTGTCAGCAGCGTAGCAACTGGCCTCCGCTCTCTAAAACGTCA  
AATAATGACAGTCGTCGCATCGTTGACCGGAGGCACACTTCGGCGAGGGACCAAGGTGGAGATCAAACGAACGTGAAAG  
CCGCCTCCCTGGTTCCACCTCTAGTTGCTTGA

**9E7 LC Light Chain Amino Acid Sequence:**

Met-Glu-Ala-Pro-Ala-Gln-Leu-Leu-Phe-Leu-Leu-Leu-Trp-Leu-Pro-Asp-Thr-Thr-Gly-  
Glu-Ile-Val-Leu-Thr-Gln-Ser-Pro-Ala-Thr-Leu-Ser-Leu-Ser-Pro-Gly-Glu-Arg-Ala-Thr-  
Leu-Ser-Cys-Arg-Ala-Ser-Gln-Ser-Val-Ser-Ser-Tyr-Leu-Ala-Trp-Tyr-Gln-Gln-Lys-Pro-  
Gly-Gln-Ala-Pro-Arg-Leu-LeuIle-Tyr-Asp-Ala-Ser-Asn-Arg-Ala-Thr-Gly-Ile-Pro-Ala-Arg-  
Phe-Ser-GlySer-Gly-Ser-Gly-Thr-Asp-Phe-Thr-Leu-Thr-Ile-Ser-Ser-Leu-Glu-Pro-Glu-Asp-  
Phe-Ala-Val-Tyr-Tyr-Cys-Gln-Gln-Arg-Ser-Asn-Trp-Pro-Pro-Leu-Thr-Phe-Gly-Gly-Gly-  
Thr-Lys-Val-Glu-Ile-Lys-Arg-Thr

FIG. 3B

9C1AHC 9C1HC	CAGGTCCAGCTGGTGCAGTCTGGGCTGAGGTGAAGAAGCCTGG CAGGTCCAGCTGGTGCAGTCTGGGCTGAGGTGAAGAAGCCTGG
9C1AHC 9C1HC	GGTGAAGGTCTCCTGCAGGGCTTCTGGAGGCACCTTCAGCAAC <u>TA</u> GGTGAAGGTCTCCTGCAGGGCTTCTGGAGGCACCTTCAGCAAC <u>CA</u>
9C1AHC 9C1HC	TCAGCTGGGCGACAGGCCCTGGACAAGGGCTTGAGTTAAT <u>AA</u> TCAGCTGGG <u>CG</u> ACAGGCCCTGGACAAGGGCTTGAGTTAAT <u>GA</u>
9C1AHC 9C1HC	ATCATCCCTATCCTTGGTATTACAAACTACGTACAGAAAGTTCCAG ATCATCCCTATCCTTGGTATTACAAACTACGTACAGAAAGTTCCAG
9C1AHC 9C1HC	AGTCACGATTACCGCGGACAGATCCACGAGCACAGCCTACATGGA AGTCACGATTACCGCGGACAGATCCACGAGCACAGCCTACATGGA
9C1AHC 9C1HC	GCAGCCTGAGATCTGAGGACACGGCCGTGTATTATTGTGCGAGAG GCAGCCTGAGATCTGAGGACACGGCCGTGTATTATTGTGCGAGAG
9C1AHC 9C1HC	AGCTACAGCTGGTTCGACCCCTGGGCCAGGGAACCTGGTCACC AGCTACAGCTGGTTCGACCCCTGGGCCAGGGAACCTGGTCACC
9C1AHC 9C1HC	CTC CTC

FIG. 4

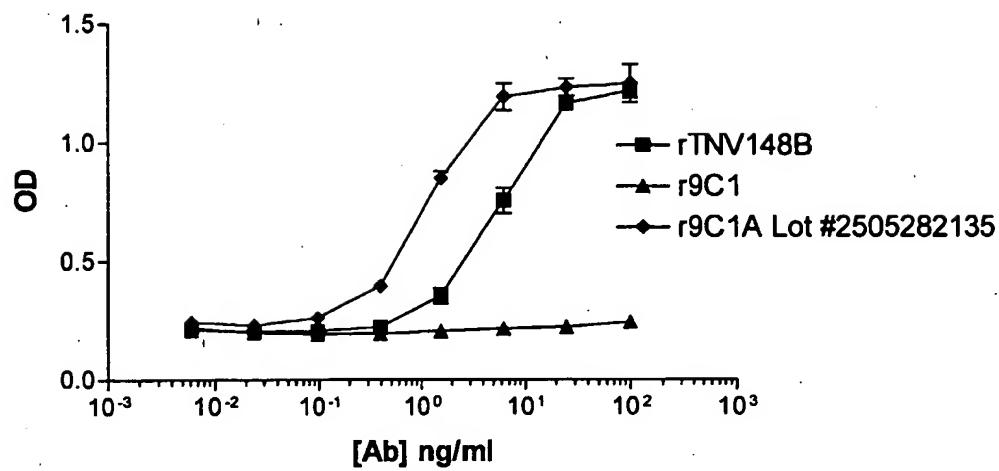


FIG. 5

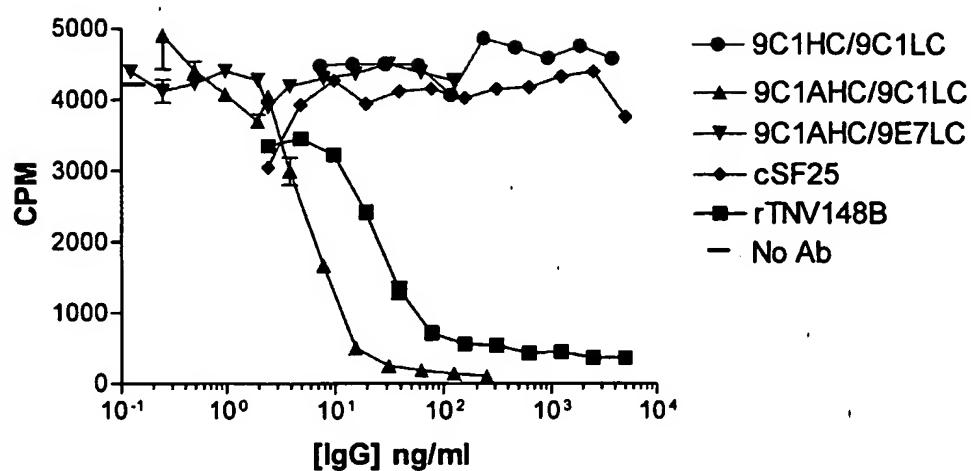


FIG. 6

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Jill Carton  
Bernie Scallon

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35 40 45

Gly Lys Ile Ile Pro Ile Leu Gly Ile Thr Asn Tyr Val Gln Lys Phe  
50 55 60

Gln Asp Arg Val Thr Ile Thr Ala Asp Arg Ser Thr Ser Thr Ala Tyr  
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Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys  
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Ala Arg Gly Gly Ser Tyr Ser Trp Phe Asp Pro Trp Gly Gln Gly Thr  
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 20 25 30

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 35 40 45

Gly Lys Ile Ile Pro Ile Leu Gly Ile Thr Asn Tyr Val Gln Lys Phe  
 50 55 60

Gln Asp Arg Val Thr Ile Thr Ala Asp Arg Ser Thr Ser Thr Ala Tyr  
 65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys  
 85 90 95

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 35 40 45

Val Ser Ser Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro  
 50 55 60

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20 25 30

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35 40 45

Val Val Pro Ser Glu Gly Leu Tyr Leu Ile Tyr Ser Gln Val Leu Phe  
50 55 60

Lys Gly Gln Gly Cys Pro Ser Thr His Val Leu Leu Thr His Thr Ile  
65 70 75 80

Ser Arg Ile Ala Val Ser Tyr Gln Thr Lys Val Asn Leu Leu Ser Ala  
85 90 95

Ile Lys Ser Pro Cys Gln Arg Glu Thr Pro Glu Gly Ala Glu Ala Lys  
100 105 110

Pro Trp Tyr Glu Pro Ile Tyr Leu Gly Gly Val Phe Gln Leu Glu Lys  
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